Survival of *Botrytis cinerea* as Mycelium in Rose Crop Debris and as Sclerotia in Soil

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ABSTRACT

Botrytis blight caused by *Botrytis cinerea* is an important disease of rose (*Rosa hybrida*) grown in greenhouses in Brazil. As little is known regarding the disease epidemiology under greenhouse conditions, pathogen survival in crop debris and as sclerotia was evaluated. Polyethylene bags with petals, leaves, or stem sections artificially infected with *B. cinerea* were mixed with crop debris in rose beds, in a commercial plastic greenhouse. High percentage of plant parts with sporulation was detected until 60 days, then sporulation decreased on petals after 120 days, and sharply decreased on stems or leaves after 90 days. Sporulation on petals continued for 360 days, but was not observed on stems after 150 days or leaves after 240 days. Although the fungus survived longer on petals, stems and leaves are also important inoculum sources because high amounts of both are deposited on beds during cultivation. Survival of sclerotia produced on PDA was also quantified. Sclerotia germination was greater than 75% in the initial 210 days and 50% until 360 days. Sclerotia weight gradually declined but they remained viable for 360 days. Sclerotia were produced on the buried petals, mainly after 90 days of burial, but not on leaves or stems. Germination of these sclerotia gradually decreased after 120 days, but lasted until 360 days. Higher weight loss and lower viability were observed on sclerotia produced on petals than on sclerotia produced *in vitro*.

Additional keywords: *Rosa hybrida*, Botrytis blight, epidemiology, management.

RESUMO

Sobrevivência de *Botrytis cinerea* como micélio em restos de culturas de rosas e como escleródios no solo

O mofo cinzento, causado por *Botrytis cinerea*, é doença importante em roseiras (*Rosa hybrida*) cultivadas em casas de vegetação no Brasil. Como pouco se conhece acerca da epidemiologia da doença nessas condições, avaliou-se a sobrevivência do patógeno em restos culturais e como escleródios. Restos de pétalas, folhas e hastes de roseira inoculados com *B. cinerea* foram colocados em sacolas de polietileno, que foram misturadas a restos culturais em canteiros de roseiras cultivadas sob estufa plástica. Observou-se alta percentagem de partes com esporulação do patógeno até 60 dias do enterrio das mesmas. A viabilidade do fungo decresceu em hastes e folhas, após 90 dias, e, em pétalas, após 120 dias. Houve esporulação em pétalas por até 360 dias, mas não em hastes ou folhas após 150 ou 240 dias, respectivamente. O patógeno sobreviveu por mais tempo em pétalas. Entretanto, hastes e folhas são importantes fontes de inóculo, por serem produzidas e depositadas em maiores quantidades nos canteiros. Quantificou-se, também, a sobrevivência de escleródios, cuja germinação foi superior a 75% e 50% nos primeiros 210 e até 360 dias, respectivamente. A massa dos escleródios reduziu, mas eles permaneceram viáveis por 360 dias. Não ocorreu produção de escleródios nas folhas e hastes enterradas, mas ocorreu nas pétalas, principalmente após 90 dias do enterrio. A germinação desses escleródios reduziu-se após 120 dias, mas estes permaneceram viáveis até 360 dias. Maior perda de massa e menor viabilidade ocorreram em escleródios produzidos nas pétalas que naqueles produzidos *in vitro*.

Palavras-chave adicionais: *Rosa hybrida*, mofo cinzento, epidemiologia, controle.

INTRODUCTION

Ornamental crops are becoming an important economic activity in Brazil due to both intra and international trade. In 2003, all direct and indirect activities related to ornamentals contributed approximately 1.5 billion dollars (IBRAFLOR, 2003) to the economy. There is an increase in the volume of ornamental plants and cut flowers being produced, both internally and for export markets (Ministério do Desenvolvimento, Indústria e Comércio Exterior, 2003). The rose (*Rosa hybrida* L.) is the most extensively cultivated cut flower crop grown for exporting. In Brazil, roses are cultivated both in the field and inside plastic greenhouses. In the greenhouse, roses are usually planted in raised beds with drip irrigation systems for watering and fertilizing.

Growers usually prune rose plants in June (winter), sprouting starts by September (spring), and production onset occurs in October. Despite intensive crop management practices, losses due to diseases can be high.

Botrytis cinerea Pers.: Fr. is a destructive pathogen of roses grown as cut flowers in Brazil and other countries (Hammer & Evensen, 1996; Tatagiba *et al.*, 1998). The pathogen colonizes petals causing lesions that reduce both yield and quality (Volpin & Elad, 1991). Economic loss frequently occurs after harvest, as undetected latent infection established during the growing season may become severe in the wet, cool, and dark conditions commonly associated with storage and transport of cut roses (Hausbeck & Moorman, 1996).

Botrytis blight control in flower crops is mostly based on fungicide sprays whose efficiency has been limited (Elad & Volpin, 1991). Resistant pathogen populations and fungicide debris are of major concern to growers. Pathogen populations that are resistant to fungicides, especially benzimidazoles and dicarboximides, are frequently reported (Hausbeck & Moorman, 1996; Oshima *et al.*, 2002). Additionally, fungicide debris cause reduction in cut flower cosmetic quality. Therefore, cultivation practices potentially effective in reducing the amount of inoculum inside the greenhouse could contribute to decreased fungicide usage (Hausbeck & Moorman, 1996; Mertely *et al.,* 2000).

Large amounts of crop debris are deposited on rose beds because of natural abscission and crop management practices in rose plant cultivation. *Botrytis cinerea* sporulates on this debris under high moisture conditions. For rose plants grown in greenhouse in Brazil, it is not known how long the fungus sporulates on rose crop debris or whether these substrates are important to pathogen survival. Furthermore, pathogen survival as mycelium or sclerotia is also unknown in Brazilian conditions.

The role of sclerotia as initial inoculum of *B. cinerea* is controversial. Sclerotia are collectively considered the most important survival structures of *B. cinerea* (Coley-Smith, 1980) and important initial inoculum for Botrytis blight epidemics (Braun & Sutton, 1987; Sutton, 1990). However, in warm-dry climates, such as Southeastern Spain, no sclerotia were found on plant debris or on living plant material, suggesting that they do not play a major role in epidemic development. In this region, *B. cinerea* commonly survived as mycelium (Raposo *et al.*, 2001). A similar situation has been reported in Israel (Elad *et al.*, 1992). Survival of *B. cinerea* sclerotia has also been studied: they could survive in the soil for five to nine months (Thomas *et al.*, 1983) and about 7% of viable sclerotia were recovered after 18 months in the soil (Hsiang & Chastagner, 1992).

There is no information on *B. cinerea* sclerotia survival in soil associated or not with rose crop debris in greenhouse under Brazilian conditions. Furthermore, no studies have been conducted on *B. cinerea* survival in rose debris to support Botrytis blight management practices such as sanitation, crop rotation, or even chemical control of

pathogen in crop debris (Jarvis, 1992). Therefore, the objective of this work was to quantify the survival of *B. cinerea* as mycelium in rose crop debris as well as sclerotia produced *in vitro* and on rose petals, under commercial greenhouse conditions.

MATERIALS AND METHODS

Survival of *Botrytis cinerea* mycelium in plant parts

This study was conducted in a commercial plastic greenhouse at Antonio Carlos, State of Minas Gerais (MG), and at the Laboratory of Epidemiology of the Department of Plant Pathology at the Universidade Federal de Viçosa, MG, from July 1993 to June 1994.

Preliminary experiments determined the most susceptible phenological stage of petals, leaves, and stems to infection by *B. cinerea*. External old petals, mature leaves, and stems at intermediate maturation stage near flower bud opening of 'Kiss' roses were used. All plant parts were inoculated with a suspension of 2×10^5 conidia/ml from a 12 day old culture of *B. cinerea* (Hammer & Marois, 1988) and incubated for 24 h in a mist chamber at 20 ºC in the dark. Sporulation was visually checked after three days.

The experiment was set in a randomized complete block design with four blocks. One rose bed was considered a block. Forty eight samples each of 40 infected petals, 48 of 40 infected leaflets, and 48 of 10x10 cm-long stem sections were prepared. Each sample was placed in a 10 x 10-cm polyethylene net bag. The bags were stapled at the ends, thoroughly mixed with crop debris, about 10 cm deep and randomly placed on beds planted with rose plants in a commercial plastic greenhouse. Twelve bags of each plant part were randomly mixed in each flower bed. Monthly, one bag of each plant part per replicate was removed and taken to the laboratory. Plant parts were separated in units (an individual leaflet, a stem segment, or a petal), placed in moist chambers (46 x 36 x 6.5 cm plastic trays, lined with wet paper towel) and kept at 20 ºC (Braun & Sutton, 1987). Mycelium survival was assessed after five days based on visual inspection under a stereomicroscope for pathogen sporulation. Viability was expressed in percentage relative to the number of units of each recovered plant material.

Survival of *B. cinerea* sclerotia

Mycelial plugs of seven day-old cultures of a *B. cinerea* isolate obtained from 'Kiss' were placed on Petri dishes containing potato dextrose agar (PDA) and incubated at 15 ºC in the dark. After 30 days, sclerotia were removed with tweezers, washed in distilled water, and placed over filter paper towels that were kept at laboratory conditions (temperature ranging from 20 to 25 ºC), where the sclerotia were allowed to air-dry, according to Ellerbrock & Lorbeer (1977). After drying, sclerotia were separated according to size, and those of about 3.0 mm diameter were grouped in lots each of ten sclerotia. Each lot was weighed and placed in a 10 x 10 cm nylon fabric bag (Hsiang & Chastagner,

1992), which was closed with nylon thread. Forty eight bags were buried at approximately 10 cm deep in four flower beds planted with 'Kiss' (four replicates). Therefore, twelve bags were buried in each flower bed.

Monthly, one bag was removed from each flower bed. In the laboratory, sclerotia were removed from the bag, counted, weighed, immersed in a 1.0% sodium hypochlorite solution for 2 min, rinsed in sterile distilled water, dried at room temperature on sterile filter paper (Ellerbrock & Lorbeer, 1977), placed on Petri dishes containing PDA supplemented with 100 ppm chloramphenicol, and incubated at 20 ºC in the dark. Sclerotia viability was determined by assessing myceliogenic germination (Coley-Smith, 1980) daily, for five days.

Recovery of sclerotia

In the experiment on survival in plant parts, sclerotia of *B. cinerea* were abundantly produced in the petals mixed with debris. These petals were collected monthly during 12 months, dried at laboratory conditions, and sclerotia were removed by rubbing the petals over sieves of 20, 60, and 120 meshes placed in cascade. Sclerotia retained in all sieves were separated from soil and plant debris. When sclerotia could not be distinguished from plant fragments, the material was placed on white paper, under a magnifying glass, and sclerotia were selectively collected with tweezers. Sclerotia removed from petals were counted and weighed. Ten sclerotia per flower bed were randomly chosen; their viability was determined in the same way as the *in vitro*-produced sclerotia described above.

Data analysis

Descriptive statistics and correlation analysis, when applicable, were carried out using SAS version 8.0 (SAS Institute, USA).

RESULTS

Survival of *Botrytis cinerea* mycelium in plant parts

Botrytis cinerea survived in all plant parts. Maximum survival was observed up to 60 days (Figure 1). The pathogen sporulated on most petals recovered up to 90 days, but the percentage of sampled petals with sporulation decreased afterward. From 120 days on, there was a trend towards stabilization while the fungus was recovered from petals until 360 days. Sporulation was visible on most stem sections up to 60 days and was not observed after 150 days (Figure 1). Pathogen sporulation was recorded on most leaves recovered up to 60 days, then the percentage of leaves with sporulation declined although some was recorded up to 240 days (Figure 1).

Survival of *B. cinerea* sclerotia

Sclerotia were abundantly produced on rose petals during the first 60 days and generally decreased after 90 days (Figure 2).

In vitro-produced sclerotia remained viable for up to 360 days in the soil (Figure 3). The viability of these sclerotia remained above 40% and was greater than viability of petal-produced sclerotia (Figure 3). Although viability was low, sclerotia weight remained constant throughout most of the assessment periods (Figure 4). Sclerotia collected at 120 days and after were lighter than the sclerotia used to set the experiments, but sclerotia germinability at both ages was similar. There was no correlation between sclerotia weight and viability (r = 0.435, P=0.138).

Germination of petal-produced sclerotia was fairly variable and decreased sharply after 120 days, but remained stable up to 360 days (Figure 3). Generally, these sclerotia lost less weight and their viability was lower than *in vitro*-produced sclerotia, but they remained viable for the same time as for *in vitro*-produced sclerotia (Figures 3 and 4). There was no evidence of association between weight and germination of petal-produced sclerotia (r = -0.388, P=0.213).

DISCUSSION

The pathogen survived on all plant parts, but with different trends. Although *B. cinerea* survived longer in petals, petals are considered a less important inoculum source than leaves and stems. It was observed that most rose buds are cut and removed from commercial greenhouses so the amount of fallen petals is always less than that for stem and leaf debris. Natural leaf abscission, accidental stem breakage, and common bedding practices such as pruning and harvesting contribute to increase the amount of stem and leaf debris on flower beds. Before setting the experiment, sclerotia were not found in naturally infected rose petals,
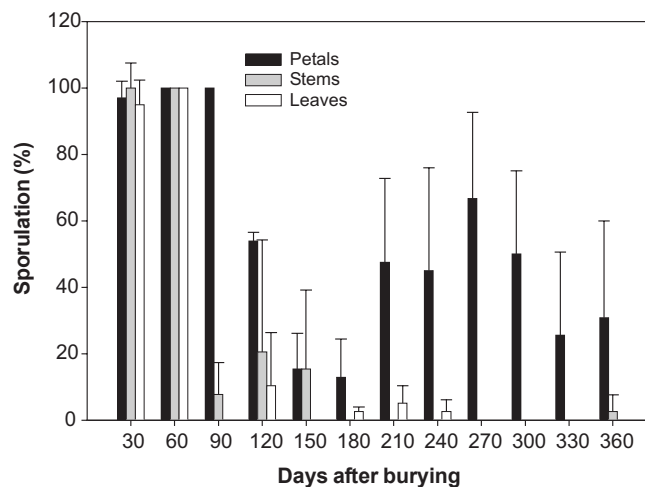

FIG. 1 - Survival of *Botrytis cinerea* on rose (*Rosa hybrida*) plant petals, stems, and leaves. Survival was estimated based on the amount of pathogen sporulation in rose organs placed in 10 x 10-cm polyethylene net bags, which were thoroughly mixed with crop debris, about 10 cm deep, on beds planted with rose plants in a commercial plastic greenhouse. Error bars represent mean standard deviation.

leaves, stems, and debris, even after exhaustive and careful examination of these materials. Interestingly, sclerotia were produced on the artificially inoculated petals which were mixed with debris.

Variation of pathogen survival in different plant parts may be related to changes in physical attributes of tissues, mainly moisture loss, caused by microclimate variations in the beds, and/or colonization by other microorganisms. In this study, plant parts remained mixed in the covering layer of crop debris on flower beds and on the top soil layer. Some samples of leaves and petals, even when collected before 120 days, were almost decomposed and had lost their individuality. Meanwhile, other samples, although in an advanced stage of dehydration, maintained their individual characteristics. Thus, *B. cinerea* survival after 90 days may have been influenced by the random distribution of plant materials in flower beds, different degrees of exposure to microclimate conditions, and interaction with microorganisms. The drip irrigation system for bed watering may have changed local microclimatic conditions, which affected both preservation of plant materials and pathogen survival. Some petal, leaf, and stem samples remained wet for prolonged periods, while others were not directly exposed to moisture. Colonies of many saprophytic microorganisms, which may have reduced *B. cinerea* sporulation, emerged from plant parts kept in moist chambers in the laboratory (data not shown). Overall, the effects of soil moisture on survival of conidia and mycelium of *B. cinerea* are not well understood (Moyano & Melgarejo, 2002). Under greenhouse conditions, higher sporulation of *B. cinerea* was observed on rose materials located on wetter parts of the debris layer. An increase in *B. cinerea* sporulation with increased humidity has been reported (O'Neill *et al.*, 1997). Mycelial survival of *B. cinerea* isolates varied from one year at 95-100% relative

humidity (RH) to less than a month at 20 °C and less than 95% RH (van den Berg & Lentz, 1968). The pathogen sporulated more abundantly on rose leaf surfaces oppositely positioned to the surfaces covered by crop debris. Even though no mycelial growth and conidia production were observed after 150 days, *B. cinerea* sporulation was observed on stem samples collected at 360 days. The lack of a quantitative relationship between the dry weight of dead strawberry plant leaves and *B. cinerea* sporulation was attributed to aggregated patterns of distribution of host tissue infected by the pathogen, differential colonization, or to pathogen survival in various debris strata (Sutton *et al.*, 1988). In the present study, the strata effects may have been implicated in the differential
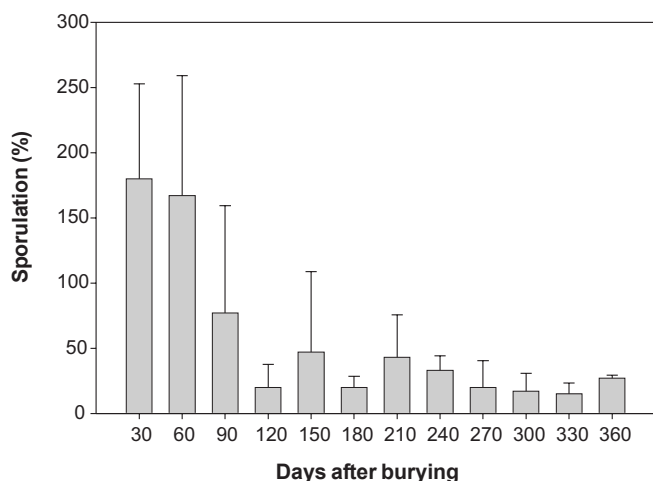

FIG. 3 - Viability of *Botrytis cinerea* sclerotia produced *in vitro* and buried in the soil, about 10 cm deep, or extracted from rose (*Rosa hybrida*) petals and deposited among crop debris, for different periods. Error bars represent mean standard deviation.

FIG. 2 - Production of *Botrytis cinerea* sclerotia on rose (*Rosa hybrida*) petals. The petals were placed in 10 x 10-cm polyethylene net bags, which were thoroughly mixed with crop debris, about 10 cm deep, on beds planted with rose plants in a commercial plastic greenhouse. Error bars represent mean standard deviation.
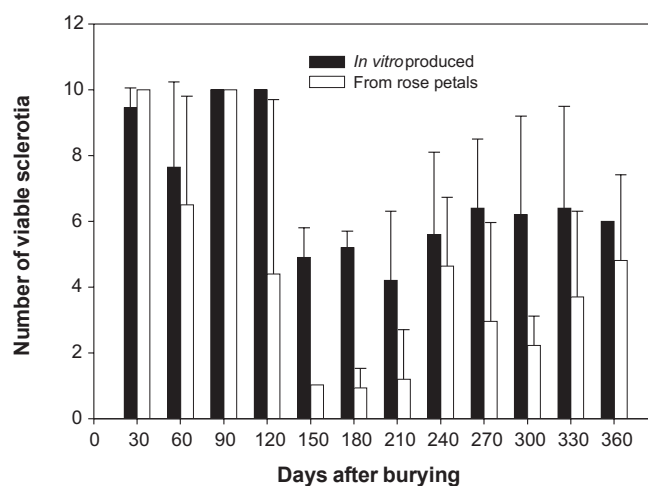

FIG. 4 - Weight of ten *Botrytis cinerea* sclerotia produced *in vitro* and buried in the soil, about 10 cm deep, or extracted from rose (*Rosa hybrida*) petals and deposited among crop debris, for different periods. Error bars represent mean standard deviation.
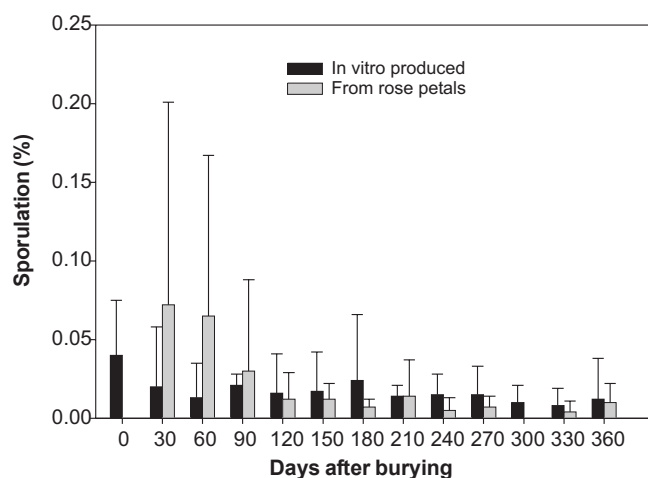
pathogen sporulation associated with leaf surface position in the litter.

Temperature variation inside the greenhouse, in the flower beds, and among crop debris may also have affected *B. cinerea* survival. Temperature extremes affect pathogen growth and sporulation, high temperatures being most harmful to both processes (Araújo, 1995). *Botrytis cinerea* survives well under low temperatures or without severe competition, but whether survival is due to mycelium or conidia is not easy to discern (Coley-Smith, 1980). In the present study, it was difficult to identify *B. cinerea* mycelium in crop debris because of tissue decomposition and colonization by other microorganisms. Although conidia viability in crop debris was not assessed, both dehydrated and recently formed conidia were found on rose materials in all evaluation periods.

In vitro-produced sclerotia remained viable for up to 360 days in the soil, although they generally lost weight over time. Fluctuations of sclerotia weight were probably due to variations in flower beds microclimate. Even though no measurements were taken, sclerotia gradually lost firmness after 120 days, which sometimes resulted in their fragmentation during extraction, but sclerotia fragments still germinated. Sclerotia viability of up to 15 months in the soil was reported (Thomas *et al.*, 1983), which is somewhat similar to the results reported here.

Germination of petal-produced sclerotia was variable and decreased after 120 days, but remained stable up to 360 days. Generally, these sclerotia lost less weight and viability than *in vitro*-produced sclerotia, but both kinds of sclerotia remained viable for the same time period. The larger reduction of viability of petal-produced sclerotia was probably because they were more exposed to temperature variations than *in vitro*-produced sclerotia which were buried in the soil.

Lower survival rates of petal-produced sclerotia may be related to their direct exposure to microclimate fluctuations in flower beds. Survival of *Botrytis squamosa* J.C. Walker sclerotia was lower when they were subjected to large fluctuations of temperature and moisture conditions (Ellerbrock & Lorbeer, 1977). In the reported experimental conditions, variations in weight and viability of sclerotia may reflect variations in bed conditions. Flower beds are exposed to direct sunlight for 60 to 90 days after winter pruning, which increases solar radiation on crop debris. Soil moisture provided by irrigation may facilitate microbial decomposition of crop debris and can affect sclerotia survival. Harvesting practices, pruning, and natural or mechanical abscission increased the accumulation of plant debris, which may modify temperature, moisture, and microbial population. As the canopy closes in the pre-harvest period, the surface of flower beds is less exposed to solar radiation leading to a potential increase in moisture level. Consequently, the changes in soil temperature and in the crop debris layer may affect both microbial activity and *B. cinerea* survival.

Apparently, there have been more favorable conditions for mycoparasitism on petal-produced sclerotia than on *in vitro*-produced sclerotia buried in the soil. Probably, nylon bags prevented thorough sclerotia-soil contact, reducing the possibility of direct action of microbial antagonists. Furthermore, buried sclerotia were subjected to less temperature fluctuations, which would have limited antagonist action during prolonged periods.

Sclerotia were not found on leaflets and stems, but sclerotia were abundantly produced on rose petals left among crop debris. However, sclerotia role as *B. cinerea* survival structures on petal debris must be better understood because they were produced only on artificially inoculated petals which were buried. Sclerotia of *B. cinerea* were rarely found on either strawberry (*Fragaria* x *ananassa* Duch.) crop debris or in the soil (Braun & Sutton, 1987).

It is important to consider some aspects of sanitation in properly managing Botrytis blight on rose plants inside greenhouses. The elimination of diseased organs, mainly flowers, from growing plants and plant materials left on flower beds and from cull piles outside greenhouses can complement other control measures. As previously stated, most flowers are harvested and taken out of the greenhouse. However, a few diseased flowers and stems, where *B. cinerea* sporulates, are not eliminated. Leaf sanitation, i.e., the removal of senescent and necrotic leaves from strawberry plants, reduced the incidence of Botrytis fruit rot (Mertely *et al.*, 2000). The same strategy could be applied to rose plants, by removing symptomatic flowers and/or stems to assure less inoculum production and dispersal in the greenhouse. As observed here, fallen petals are an efficient inoculum source for the pathogen.

Although *B. cinerea* survival was less on stems and leaves, the continuous presence of their debris, whether from crop management practices or natural senescence, may guarantee pathogen survival in greenhouses for one cycle or between crop cycles. Stems and leaves recently deposited on the soil would require more attention. Growers prune rose plants in winter, sprouting starts in the spring, and production onset occurs right after. Therefore, in two to three months time span, *B. cinerea* survival is assured regardless of the inoculum source. It is important to adopt disease management practices, such as eliminating crop debris aimed at reducing substrate availability for *B. cinerea* sporulation. The physical removal or destruction of infected plant parts and crop debris, recommended for *Botrytis* spp. management in greenhouse-grown flowers (Jarvis, 1992; Hausbeck & Moorman, 1996), should also be applied to rose crops.

After pruning, diseased and non-diseased parts of rose plants are usually left outside greenhouses in cull piles. This plant debris should be destroyed (Moyano & Melgarejo, 2002), because the mycelium outside greenhouses that has probably survived in infected plant debris is a primary inoculum source of *B. cinerea* (Raposo *et al.*, 2001).

Abundant sclerotia production was observed in artificially inoculated petals which were mixed with crop debris in the flower beds. Sclerotia are considered important primary inoculum for Botrytis blight epidemics (Braun &

Sutton, 1987; Sutton, 1990). However, no sclerotia of *B. cinerea* were found in Spain (Raposo *et al.*, 2001) or Israel (Elad *et al.*, 1992). Under the conditions in which this research was conducted, sclerotia are not considered as an important inoculum source of *B. cinerea* in part because they were not found but mainly because there is a constant supply of infected diseased tissue being deposited on the flower beds year round.

Sanitation practices aimed at reducing both survival and sporulation of *B. cinerea* should be conducted to reduce initial inoculum and delay Botrytis blight epidemics in rose plants cultivated in greenhouses. However, it is important to note that Botrytis blight is a polycyclic disease. Thus, successful management of Botrytis blight must also include other practices that suppress secondary inoculum buildup to reduce the disease progress rate.

ACKNOWLEDGEMENTS

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