

Biological Control of *Botrytis cinerea* in Residues and Flowers of Rose (*Rosa hybrida*)

Joseli da Silva Tatagiba,¹ Luiz Antonio Maffia,¹ Roberto W. Barreto,¹
Acelino C. Alfenas¹ and John C. Sutton²

Microbial isolates from living petals, petal residues and leaf residues of rose, and from laboratory collections, were evaluated for control of *Botrytis cinerea* in rose. In leaf residues artificially infested with *B. cinerea*, isolates of the filamentous fungi *Gliocladium roseum*, FR136 (unidentified) and *Trichoderma inhamatum* reduced sporulation of the pathogen by >90%, other filamentous fungi were 25–90% effective, and those of yeasts and bacteria were <50% effective. In artificially inoculated petal residues, no microbe reduced sporulation of *B. cinerea* by >75%, but isolates of *Cladosporium oxysporum* and four yeasts were 51–75% effective, and three filamentous fungi, eight yeasts and *Bacillus subtilis* isolates were 26–50% effective. Isolates of *T. inhamatum*, *C. oxysporum* and *G. roseum* performed best against *B. cinerea* among isolates evaluated in leaf residues naturally infested with the pathogen and indigenous microorganisms. Totals of ten isolates of filamentous fungi (including *C. oxysporum* and *C. cladosporioides*), two of yeasts and five of *Bacillus subtilis* completely prevented lesion production by *B. cinerea* in detached petals, and a further six isolates of filamentous fungi (including *G. roseum*) and six yeasts were 90–99% effective. Isolates of *C. oxysporum*, *C. cladosporioides* and *B. subtilis*, the most effective microorganisms against *B. cinerea* in flower buds, reduced number of lesions in the range of 42–65% compared with 59–89% for a standard fungicide (vinclozolin). It is suggested that application of leading antagonists to living rose leaves and flowers should optimize control of inoculum production by *B. cinerea* when the tissues die. Optimal biocontrol of lesion production in flower buds requires a better understanding of the microenvironment of petals.

KEY WORDS: Rose; *Botrytis cinerea*; biological control.

INTRODUCTION

Botrytis cinerea Pers.: Fr. is one of the principal causes of pre- and postharvest losses in roses (*Rosa hybrida* L.) produced in greenhouses in the Brazilian States of Minas Gerais and São Paulo. In Brazil, as elsewhere (3,10,18,29), the pathogen produces spreading necrotic lesions on leaves, shoots and renewal canes (epicormic shoots) in the greenhouse, and small lesions and blighting in petals of cut flowers in storage or transit. Economic losses are associated chiefly with symptoms that develop in petals of harvested flowers at sites where the pathogen was quiescent or latent when the flower buds were cut (3,10). Thus, postharvest disease results from inadequate control of *B. cinerea* when roses are produced in the greenhouse.

Received April 14, 1997; received in final form Aug. 4, 1997.

¹Depto de Fitopatologia, Universidade Federal de Viçosa, 36570 Viçosa, Minas Gerais, Brazil [Fax: +31-8992240; e-mail: lmaffia@mail.ufv.br].

²Dept. of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1.

Sanitation, cultural practices and chemical fungicides are used as control measures against *B. cinerea* in rose production systems in Brazil, but growers depend chiefly on fungicides (1,28). Effective resistance against *B. cinerea* is lacking in rose cultivars (17). The pathogen is readily introduced into new rose plantings from sources within and outside the greenhouse. A majority of the greenhouses used for rose production in Brazil are polyethylene-covered structures, often with sides and ends partially opened, and lacking sophisticated control of microclimate. Inoculum of *B. cinerea* is easily blown into these greenhouses, and regulation of microclimatic variables such as atmospheric vapor pressure deficit to suppress development of the pathogen is not feasible. Increased air circulation and judicious timing of irrigation to minimize periods of high humidity and leaf wetness, and sequencing of crops to minimize inoculum carryover, might suppress the pathogen in some greenhouses but these techniques have not been investigated scientifically. Benzimidazole and dicarboximide fungicides, widely used in Brazil to control *B. cinerea* in roses, have declined markedly in effectiveness in many greenhouses, apparently because populations of the pathogen have become increasingly insensitive, as frequently reported elsewhere (8,11,30).

Microbial antagonists of *B. cinerea* might have potential for improving control of the pathogen in rose crops. Redmond *et al.* (19) identified isolates of yeasts and bacteria that reduced numbers of lesions produced by the pathogen on rose petals, and Elad *et al.* (6) observed that *Trichoderma harzianum* reduced symptoms on rose flowers that were harvested soon after treatment. Hammer and Marois (7) successfully used two biological agents to reduce symptoms caused by *B. cinerea* in rose flowers stored at 2.5°C, but control was ineffective when the flowers were removed from storage and kept at room temperature (21°C). Biological control of *B. cinerea* has not been reported in leaves, stems and residues of roses, which are important inoculum sources of the pathogen for flower infection of roses in greenhouses (1). However, strong suppression of *B. cinerea* by microbial agents was reported in living and dead foliage as well as in flowers and fruits of various other ornamental plants, and of vegetables and fruit crops, in greenhouses and outdoors (13,16,23-26,32). In the present study, microbial isolates were examined for the ability to suppress spore production of *B. cinerea* in rose crop residues and to protect rose flowers against the pathogen.

MATERIALS AND METHODS

Isolation of microorganisms

Flowers, petal residues and leaf residues of rose were collected in greenhouses and outdoor plantings in Antônio Carlos, Viçosa, Paraíso, Machado, Juquinha de Paula and Córrego da Agonia in Minas Gerais State in August and September 1994. To recover microorganisms, detached petals and residues of petals and leaves were shaken in sterile distilled water in Erlenmeyer flasks for 10 min. Portions of wash water in each flask were serially diluted and 0.1-ml aliquots were spread on potato dextrose agar (PDA) medium with or without 200 ppm tetracycline added. Other portions were incubated in a water bath at 80°C for 20 min prior to plating on PDA media to favor isolation of *Bacillus* spp. (22). Microbial isolates were obtained from colonies that formed on the media after the petri dishes were incubated at 25°C in darkness for up to 20 days. The isolates obtained were maintained on PDA at 4°C.

Identification of isolates

Selected isolates of filamentous fungi were identified from morphological characteristics at the International Mycological Institute, England, and those of yeasts were identified at the Instituto de Microbiologia of the Universidade Federal de Rio de Janeiro. Several bacterial isolates were identified as *Bacillus* sp. based on Gram staining, catalase production and endospore formation (14,20) and further identified to species at the Fundação Tropical de Pesquisas e Tecnologia “André Tosello”, Campinas, São Paulo.

Inoculum production

Microbial isolates for evaluation in biocontrol tests, and an isolate of *B. cinerea* from rose petals, were grown on PDA in petri dishes. Filamentous fungi were cultured on PDA amended with 200 ppm tetracycline in a 12-h photoperiod at 25°C for 3 days and bacteria in darkness at 28°C for 1 day. Propagules of organisms were suspended in sterile-distilled water containing surfactant (Tween 20, final concentration 0.05%) and diluted to 10⁴, 10⁵ or 10⁶ conidia/ml for *B. cinerea*, 10⁷ conidia/ml for filamentous antagonists, 10⁷ cells/ml for yeasts, and 10⁸ cells/ml for bacteria, unless otherwise indicated. Concentrations of fungal propagules and bacterial cells were estimated with the aid of a Neubauer chamber and the MacFarland scale, respectively (12).

Humidity chambers

Clear plastic boxes (11 cm × 11 cm × 3.5 cm deep) with lids were used as humidity chambers in biocontrol tests. In the tests, tissue disks were placed on nylon screening (2 mm mesh) that overlaid 1-cm-thick sponge foam which was moistened with water.

Biocontrol in artificially infested rose residues

Twenty-seven isolates of filamentous fungi, nineteen of yeasts and two of *Bacillus* spp. from rose residues, one isolate each of *Gliocladium roseum* Bainier and *Trichoderma longibrachiatum* Rifai, and two isolates of *Trichoderma inhamatum* Veerkamp and W. Gams, were evaluated for the ability to suppress *B. cinerea* previously established in dead leaf and petal tissues. The isolate of *G. roseum* was from the University of Guelph, Canada, and the isolates of *Trichoderma* were from the Universidade de Viçosa, Brazil. One-cm-diameter disks of green leaves and fresh petals of rose cv. ‘Kiss’, that were not surface-sterilized, were inoculated with *B. cinerea* (10⁶ conidia/ml) by means of a small hand sprayer (De Vilbiss No. 15) and placed on moistened paper towels on plastic trays inside clear plastic bags. The bags were sealed to maintain high humidity and kept at 20°C for 3 and 7 days for petals and leaves, respectively, at which times lesions had developed. The disks were allowed to dry slowly for 7 to 10 days to simulate formation of crop residues. The dried disks were immersed twice in inoculum suspensions of the microbial antagonists, each time for 2 sec, and placed in the humidity chambers. There were three replicate chambers each with 12 disks per treatment. Disks were incubated in the chambers for 24 h in darkness at ~25°C and subsequently in a growth cabinet beneath fluorescent lamps (24-h photoperiod; 2100 lux at chamber height) at 20°C for 3 days (petals) and 7 days (leaves). After incubation, percent area of each disk with conidiophores of *B. cinerea* was estimated visually on a dissecting microscope. The scale used to facilitate estimations, based on that of Horsfall and Barratt (9), was as follows: 0=0%, 1=>0–25%, 2=>25–

50%, 3=>50–75%, 4=>75–<100%, and 5=100% of disk area with conidiophores of *B. cinerea*. From these data, a disease index (DI) in percent (15) was calculated according to the equation:

$$DI = \frac{\sum(f \cdot n)}{MN \cdot no} \cdot 100, \quad (1)$$

where f = frequency of a note, MN = maximum note and no = number of observations.

Biological control in naturally infested residues

Seven isolates of filamentous fungi of superior effectiveness in suppressing *B. cinerea* in artificially infested residues were evaluated against the pathogen in fallen dead leaves of rose that were collected arbitrarily in greenhouses near Antônio Carlos. The leaves were examined with a hand lens and those bearing conidiophores of *B. cinerea* were selected for biocontrol treatments. Selected leaves were transported in plastic bags to the laboratory, where they were agitated manually for 5 min to remove conidiophores of the pathogen. Disks 0.8 cm in diameter were cut from the leaves, examined on a dissecting microscope to confirm the absence of conidiophores, and placed in the humidity chambers. The disks were sprayed with inoculum of the test isolates, and incubated and evaluated as described for artificially infested disks. There were 20 disks in each of three replicated chambers per treatment. The experiment was a completely randomized design and was repeated once.

Biocontrol in petals

Sixty-seven microbial isolates from rose petals and isolates of *G. roseum*, *T. longibrachiatum* and *T. inhamatum* examined in rose residues were evaluated against *B. cinerea* in four separate tests. *G. roseum* was employed as the standard antagonist in each test. One-cm-diameter disks cut from petals of rose cv. Kiss were placed in humidity chambers. The surface of each disk was inoculated with a 10 μ l droplet of antagonist inoculum or of water plus surfactant only, and immediately, at the same site, with a 10 μ l inoculum droplet of *B. cinerea*. In this study, conidia of the pathogen (10^5 spores/ml) were suspended in 0.1M glucose plus 0.05M monobasic potassium phosphate to promote infection (4). The humidity chambers with inoculated disks were maintained in a growth chamber at 20°C in continuous light (2100 lux). At 3 days after inoculation, the incidence of petal disks with a lesion of larger diameter than the inoculum droplet was estimated. There were 25 disks in each of two humidity chambers per treatment. The experiment was a completely randomized design, and was repeated once.

Biocontrol in flower buds

Sixteen microbial isolates and the standard fungicide vinclozolin (0.75 g a.i./l) were compared for effectiveness in suppressing development of lesions caused by *B. cinerea* in flower buds of rose cv. Kiss. Rose stems, each bearing a flower bud, were cut and placed in plastic vases containing tap water. Immediately before stems were cut, two outermost petals of each bud were removed because of possible latent infection by *B. cinerea*. All treatments were applied to all outer surfaces of flower buds by means of a small sprayer (DeVilbiss No. 15), in a series of four tests in which each isolate was evaluated twice. Buds were treated with microbial antagonists, with vinclozolin, or with water plus surfactant, and each was covered with a plastic bag to serve as a humidity chamber. Inoculum concentration of microbial isolates was as indicated previously except

for FP139, FP157 and FP168, which were applied at 1×10^5 , 5×10^5 and 6×10^6 conidia/ml, respectively, in test 2, and at 4×10^5 , 5×10^6 and 3×10^6 conidia/ml, respectively, in test 3. After 24 h in high humidity at room temperature (20–25°C), the buds were inoculated with *B. cinerea* (10^4 conidia/ml), covered with plastic bags, and maintained in a growth cabinet at 20°C. The bags were removed at 24 h, and lesions were counted on all flower buds 48 h later. The experiment was a completely randomized design with three replicate vases each containing eight stems, each with one flower bud, per treatment. The study was repeated once.

Data analysis

Statistical computations were performed using the Sistema de Análises Estatísticas e Genéticas (Universidade Federal de Viçosa, Viçosa, Brazil). Data were examined using the procedures for general analysis of variance (ANOVA). Observations of repeated experiments were subjected to analyses of homogeneity of variance and pooled accordingly. Cluster analysis (21) was used to evaluate differences among treatment means.

RESULTS

Biocontrol in artificially infested residues – Leaves. *Botrytis cinerea* sporulated on 88% of residue area in check tissues that were inoculated with the pathogen when green, kept under conditions conducive to infection and colonization, dried, and treated with water plus Tween 20. A majority of microbial isolates reduced the area of sporulation by the pathogen, but only the filamentous fungi *G. roseum*, FR136 and *T. inhamatum* UFV3 did so by >90%, and among these only *G. roseum* was 100% effective (Table 1). All isolates that reduced sporulation of *B. cinerea* to less than 50% of residue area were filamentous fungi. Yeasts, bacteria and other filamentous fungi were <50% effective.

– **Petals.** *Botrytis cinerea* sporulated on the entire upper surface of check residues that were inoculated with the pathogen as fresh petal disks, kept under conditions conducive to infection and colonization, dried, and treated with water plus surfactant. A majority of microbial isolates tested failed to reduce sporulation of the pathogen to 75% of residue area (Table 2). Thirteen isolates reduced sporulation to 51–75% of residue area, and five did so to 26–50%, but none was more effective. Among the 18 leading isolates 12 were yeasts, four were filamentous fungi and two were bacteria.

Biocontrol in naturally infested leaves. Among the isolates tested, *T. inhamatum* (UFV3), *Cladosporium oxysporum* (FR133) and *G. roseum* significantly reduced area of residues with sporulation of *B. cinerea* in both repetitions of the study (Table 3). Other isolates suppressed *B. cinerea* in one of the two repetitions.

Biocontrol in petals. Lesions developed in all petal disks treated with water plus surfactant and inoculated with *B. cinerea*. Isolates of three filamentous fungi, two yeasts and five bacteria completely suppressed production of lesions by the pathogen, and a further six filamentous fungi and six yeasts suppressed lesions by 90–99% (Table 4).

TABLE 1. Area of sporulation of *Botrytis cinerea* in leaf-disk residues of rose that were inoculated with the pathogen when green and challenge-inoculated with isolates of microorganisms after the tissues were killed by desiccation

Area of disks with sporulation of <i>B. cinerea</i> (%)	Isolates of microorganisms ^z
0	<i>Gliocladium roseum</i>
1-5	None
6-10	FR136, <i>Trichoderma inhamatum</i> UFV3
11-25	FR142
26-50	FR18, FR19, FR29, FR88 (<i>Cladosporium</i> sp.), FR132, FR133 (<i>Cladosporium oxysporum</i>), FR141, FR145, <i>Trichoderma inhamatum</i> UFV2
51-75	FR23, FR24, FR25, FR26, FR93, FR97, FR101, FR144, FR159, <i>Trichoderma longibrachiatum</i> UFV1, LR5, LR95, LR111, LR113, LR116, LR119, LR121, LR134, LR135, LR137, LR143, LR158, BR162 (<i>Bacillus</i> sp.), BR163 (<i>Bacillus</i> sp.)
>75	FR22, FR27, FR96, FR100, FR105, FR108, FR115, FR122, LR2, LR4, LR13, LR84, LR90, LR91, LR92, check (water plus surfactant)

^zMicroorganisms designated FR, LR and BR are isolates of filamentous fungi, yeasts and bacteria, respectively, from rose residues.

TABLE 2. Area of sporulation of *Botrytis cinerea* in petal-disk residues of rose that were inoculated with the pathogen when freshly cut, and challenge-inoculated with isolates of microorganisms after the tissues were killed by desiccation

Area of disks with sporulation of <i>B. cinerea</i> (%)	Isolates of microorganisms ^z
0-25	None
26-50	FR133 (<i>Cladosporium oxysporum</i>), LR5, LR116, LR137, LR158
51-75	FR29, FR93, FR132 (<i>Pestalotiopsis</i> sp.), LR13, LR113, LR119, LR121, LR134, LR135, LR143, BR162, BR163
>75	<i>Gliocladium roseum</i> , <i>Trichoderma inhamatum</i> UFV2, <i>T. inhamatum</i> UFV3, <i>Trichoderma longibrachiatum</i> UFV1, FR18, FR19, FR22, FR23, FR24, FR25, FR26, FR27, FR88 (<i>Cladosporium</i> sp.), FR96, FR97, FR100, FR101, FR105, FR108, FR115, FR122, FR136, FR141, FR142, FR144, FR145, FR159, LR2, LR4, LR84, LR90, LR91, LR92, LR95, LR111, check (water plus surfactant)

^zIsolates designated FR, LR and BR are filamentous fungi, yeasts and bacteria, respectively, from rose residues.

Isolates of *C. oxysporum*, *Cladosporium cladosporioides*, *G. roseum* and *Bacillus subtilis* (BP154, BP155, BP161, BP164, AP3) were among the antagonists that suppressed lesions by >90%. Isolates of *Trichoderma* spp. were ineffective to moderately effective.

TABLE 3. Estimated effectiveness of fungal isolates in reducing sporulation of *Botrytis cinerea* in rose leaf residues that were naturally infested with the pathogen

Fungal isolates	Area of leaf disks with sporulation of <i>B. cinerea</i> (%)	
	Repetition 1	Repetition 2
None (water plus surfactant)	67a ^z	59a
FR136	62a	43b
FR142	56a	37b
<i>Pestalotiopsis</i> sp. (FR132)	51b	53a
<i>Trichoderma inhamatum</i> (UFV3)	51b	45b
<i>Cladosporium</i> sp. (FR88)	48b	52a
<i>Cladosporium oxysporum</i> (FR133)	45b	40b
<i>Gliocladium roseum</i>	38b	38b

^zWithin a column, values assigned the same letter do not differ significantly ($P_{0.05}$, cluster analysis; CV values, repetition 1 = 13.4, repetition 2 = 12.9).

TABLE 4. Incidence of rose petal disks with symptoms produced by *Botrytis cinerea* after the disks were inoculated with various microorganisms or with water only, and immediately inoculated with the pathogen^z

Incidence of petal disks with symptoms (%)	Isolates of microorganisms ^y
0	FP123 <i>Cladosporium oxysporum</i> , FR139 <i>Cladosporium cladosporioides</i> , FP168, LP3, LP58, BP154, BP155, BP161, BP164, AP3
1-5	FP21 <i>Cladosporium cladosporioides</i> , FP157, <i>Cladosporium oxysporum</i> , LP151, LP153, LP167
6-10	<i>Gliocladium roseum</i> , FP31, FP52, G31, LP69, LP140, LP150
11-25	FP71, G63, LP1, LP37, LP41, LP59, LP63, LP76, LP125, LP128, LP169, LP170
26-50	FP16, FP17, FP147, FP152, G41, G106, <i>Trichoderma inhamatum</i> UFV3, LP34, LP46, LP68, LP75, LP166, LP172, LP173
51-75	FP36, FP39, FP65, FP70, FP78, G108, PC, LP32, LP127
>75	FP38, FP40, FP44, FP47, FP50, FP51, FP53, FP61, FP62, FP77, FP88, FP124, FP130, FP148, <i>Trichoderma longibrachiatum</i> UFV1, <i>Trichoderma inhamatum</i> UFV2, check

^zConidia of the pathogen were suspended in 0.1M glucose plus 0.05M monobasic potassium phosphate.

^yIsolates designated as FP, G and PC are filamentous fungi, LP isolates are yeasts, and BP and AP isolates are of *Bacillus subtilis*. All FP, G, LP and BP isolates were from rose petals.

Biocontrol in flower buds. Tested microorganisms in most instances suppressed numbers of lesions produced by *B. cinerea* on rose buds but less effectively than did vinclozolin. *B. subtilis* (BP161) and *C. oxysporum* (FP123) were as effective as vinclozolin in tests 2 and 3, respectively, but this performance was not sustained in test 4. Organisms in the most effective statistical clusters generally reduced numbers of lesions in the range of 42–65%, compared with 59–89% for vinclozolin. Isolates of *C. oxysporum* (FP123 and FP157), *C. cladosporioides* (FP21, FP139) and *B. subtilis* (BP161) were leading performers except when the inoculum concentration used was much reduced (test 3, FP139

and FP157). Different isolates of *B. subtilis* varied widely in effectiveness.

TABLE 5. Effects of various microorganisms, vinclozolin, or water plus surfactant on number of lesions produced by *Botrytis cinerea* in rose flower buds^z

Treatments	Number of lesions per flower				Mean suppression of lesions (%) ^y
	Test 1	Test 2	Test 3	Test 4	
None (water check)	49a ^x	87a	127a	134a	–
<i>Bacillus subtilis</i> (BP154)	–	48b	–	116b	29
<i>B. subtilis</i> (AP3)	–	62b	–	107b	24
<i>Candida</i> sp. (LP153)	43a	–	–	105b	18
<i>B. subtilis</i> (BP155)	–	53b	–	100b	31
<i>Cladosporium</i> sp. (FP168)	–	–	76c	96c	34
<i>Sporobolomyces</i> sp. (LP3)	36a	–	–	94c	28
<i>B. subtilis</i> (BP164)	–	53b	–	91c	38
<i>Gliocladium roseum</i>	–	–	92b	83c	33
<i>Cryptococcus</i> sp. (LP58)	28b	–	–	79c	42
<i>Rhodotorula</i> sp. (LP151)	28b	–	–	79c	42
<i>Cryptococcus</i> sp. (LP167)	39a	–	–	78c	32
<i>B. subtilis</i> (BP161)	–	35c	–	72d	53
<i>Cladosporium oxysporum</i> (FP123)	–	–	57d	68d	52
<i>Cladosporium cladosporioides</i> (FP139)	–	–	109a	68d	32
<i>C. cladosporioides</i> (FP21)	–	–	74c	57d	49
<i>C. oxysporum</i> (FP157)	–	–	88b	46d	52
Vinclozolin	12c	23c	52d	15e	74
CV values	21.3	15.3	14.0	14.6	

^zFlower buds were inoculated with *B. cinerea* 24 h after treatments were applied.

^yMean values of two tests.

^xWithin a column, values assigned the same letter do not differ significantly ($P_{0.05}$, cluster analysis).

DISCUSSION

Microorganisms were identified that markedly suppressed *B. cinerea* in leaf and petal residues of rose, and symptom production by the pathogen in petals of rose buds, but the kinds of effective organisms differed in the various tissues. In residues of leaves that were inoculated with *B. cinerea* when green, several filamentous fungi, notably *G. roseum*, FR136 and *T. inhamatum* UFV3, were extremely effective against the pathogen, whereas isolates of yeasts and *Bacillus* sp. were slightly effective to ineffective. In residues of petals, previously inoculated with *B. cinerea* when freshly removed from flowers, several yeasts and an isolate of *C. oxysporum* (FR133) were leading antagonists of *B. cinerea*, unlike *G. roseum* and *T. inhamatum* that were only slightly suppressive. Isolates of *Cladosporium* spp., *B. subtilis* and some yeasts strongly suppressed *B. cinerea* in assays on fresh petal disks, and several of these isolates also performed well against the pathogen on flower buds; yeasts and most isolates of *B. subtilis*, however, were inferior to most isolates of *Cladosporium* when tested on flower buds. The observations indicated that the kind and state (living or dead) of host tissue markedly affected ranking of isolates in terms of effectiveness against *B. cinerea*. Other important variables affecting biocontrol likely included inoculum concentration of the antagonists, concentration and developmental stage of the pathogen in the host, time and method of inoculation of

antagonist and pathogen, and environmental water such as droplet size or film depth on living host tissues and water potential of dead tissues. Ecological adaptation of microbes to host tissues, including nutritional relationships, can be of critical importance in biological control (25,31).

Gliocladium roseum, *T. inhamatum* UFV3 and isolate FR136 have strong potential for controlling inoculum production of *B. cinerea* in rose leaf residues. Applied to artificially inoculated leaves, the antagonists suppressed sporulation of *B. cinerea* by 90–100% compared with water controls, in which 88% of residues were covered by conidiophores of the pathogen. Plausibly, the antagonists reduced sporulation by suppressing colonization of the residues by the pathogen, possibly through competitive colonization of the substrate. It may be presumed, given the experimental conditions, that *B. cinerea* infected the inoculated green leaves, but the extent of colonization when the leaves were dried and the antagonists were applied is not known. Substrate competition following this stage would be consistent with earlier observations on mode of biocontrol of *B. cinerea* by *G. roseum*, *Trichoderma* sp. and other antagonists in leaves of other hosts (5,13,23,27). Evidence that antagonists are able to suppress *B. cinerea* in tissues heavily colonized by the pathogen is lacking.

The reduced effectiveness of antagonists against *B. cinerea* in residues of naturally infected leaves from greenhouses probably resulted from inability to displace *B. cinerea* in tissues already colonized by the pathogen and various saprotrophic microbes. Interference in biocontrol by indigenous saprotrophs is well known (13,26). Application of antagonists to leaves before the time of senescence and death would avoid prior colonization of the tissues by the pathogen and by indigenous saprotrophs, and improve prospects for effective biocontrol, given antagonists of suitable ecological adaptation and favorable microclimate (23,27). Antagonists with ability to develop as nonpathogenic endophytes, such as *G. roseum*, appear especially well suited (25).

The generally lower effectiveness and widely differing performance of microbial antagonists against *B. cinerea* in petal residues compared to leaf residues underscored a profound effect of the plant organs on biological control. Even the most effective organisms failed to confine sporulation of the pathogen to <25% of petal residues. Further, microbial isolates that strongly suppressed *B. cinerea* in leaf residues were only slightly suppressive or nonsuppressive in petal residues. A possible factor contributing to reduced biocontrol effectiveness was greater susceptibility of petals to *B. cinerea* such that infection and colonization by the pathogen were more advanced than in leaves at the time when antagonists were applied, thereby producing a competitive advantage for *B. cinerea* in the freshly formed petal residues. As in leaves, application of antagonists to living petals might improve suppression of *B. cinerea* after the petals senesce and die.

Several microbial isolates, including filamentous fungi, yeasts and bacteria, suppressed symptoms caused by *B. cinerea* in freshly cut rose petals by 95–100%, yet were only 42–65% effective in whole flower buds of rose. Effectiveness of the microbes in flower buds was similar to that of a black yeast (*Exophiala jeanselmei*) and a coryneform bacterium, which reduced disease by 63% and 48%, respectively, in an earlier study (19). Differences in test conditions on the detached petals and whole flowers in the present study should hold the key to optimizing biological control of *B. cinerea* in rose flowers. Observations in petals underscored the potential feasibility of controlling latent infections on flower buds, and consequently symptom development prior to harvest, and in postharvest storage and

shipping. Factors of possible importance in biocontrol include nutrients used in the petal assay (glucose and monobasic potassium phosphate); method of application and resulting distribution of the antagonist and pathogen on rose tissues; potential shifts in population density of antagonists after application to rose flowers; and form (droplets, films), amounts and persistence of liquid water on treated petals. Studies of these and other relevant variables in relation to biological control should allow improved management of *B. cinerea* in rose flowers.

The leading antagonists of *B. cinerea* provide an opportunity to develop integrated disease management in rose production systems in Brazil, involving biological control of inoculum production of *B. cinerea* in rose residues and protection of flower buds against infection by the pathogen. Organisms that justify study for reducing initial inoculum of the pathogen in leaf and petal residues are *G. roseum*, *T. inhamatum* UFV3, *C. oxysporum* (FR133) and isolate FR136. Those justifying tests on flower buds include *B. subtilis* (BP161), *C. oxysporum* (FP123 and FP157), *C. cladosporioides* (FP21), *Cryptococcus* sp. (LP58) and *Rhodotorula* sp. (LP151). Strong performance of antagonists on plant organs used in biocontrol assays does not necessarily imply strong performance under epidemiologic conditions in crops, but, in strawberry, performance of microbes against *B. cinerea* in detached tissues correlated well with that in field plots (16). Important factors affecting biological control in rose crops probably include quantitative and spatiotemporal relationships among antagonist populations, rose plants, inoculum sources and dispersals of *B. cinerea*, other microbes, microclimatic variables including humid periods and temperature, and human activities, chiefly crop production and protection practices (2,8,11,24). Besides being effective, biocontrol must also satisfy requirements such as lack of pathogenicity of antagonists and absence of visible residues of microbes in treated rose flowers. Additional challenges will be to integrate biological control of *B. cinerea* with other practices used to produce roses and protect them against diseases and insects, and to develop the best antagonists commercially.

ACKNOWLEDGMENTS

Contributions of Dr. John David of the International Mycological Institute, England, in identifying filamentous fungi, of Prof. Allen Hagler, Instituto de Microbiologia, Universidade Federal de Rio de Janeiro in identifying yeasts, and of Dra. S.Y. Eguchi, Fundação Tropical de Pesquisas e Tecnologia “André Tosello”, Campinas, São Paulo, are gratefully acknowledged. We thank João Miranda dos Santos and Pedro Paulo Gonçalves for helpful advice. The research was funded in part by FAPEMIG (Fundação de Apoio a Pesquisa do Estado de Minas Gerais) and by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

REFERENCES

1. Araújo, A.E. (1995) Sobrevivência de *Botrytis cinerea* em restos de cultura, efeito de fatores do ambiente sobre o patógeno e progresso do mofo cinzento em roseiras cultivadas em casas-de-vegetação. Dissertação (mestrado). Universidade de Viçosa, Minas Gerais, Brasil.
2. Berger, R.D. (1988) The analysis of effects of control measures on the development of epidemics. pp. 137-151. *in*: Kranz, J. and Rotem, J. [Eds.] Experimental Techniques in Plant Disease Epidemiology. Springer-Verlag, Berlin.
3. Elad, Y. (1988) Latent infection of *Botrytis cinerea* in rose flowers and combined chemical and physiological control of the disease. *Crop Prot.* 7:361-366.

4. Elad, Y. (1989) Effect of abiotic conditions on development of grey mold of rose and scanning electron microscopy. *Phytopathol. Mediterr.* 28:122-130.
5. Elad, Y. (1996) Mechanisms involved in the biological control of *Botrytis cinerea* incited diseases. *Eur. J. Plant Pathol.* 102:719-732.
6. Elad, Y., Kirshner, B. and Gotlib, Y. (1993) Attempts to control *Botrytis cinerea* on roses by pre- and postharvest treatments with biological and chemical agents. *Crop Prot.* 12:69-73.
7. Hammer, P.E. and Marois, J.J. (1989) Nonchemical methods for postharvest control of *Botrytis cinerea* on cut roses. *J. Am. Soc. Hortic. Sci.* 114:100-106.
8. Hausbeck, M.K. and Moorman, G.W. (1996) Managing *Botrytis* in greenhouse-grown flower crops. *Plant Dis.* 80:1212-1219.
9. Horsfall, J.G. and Barratt, R.W. (1945) An improved grading system for measuring plant disease. *Phytopathology* 35:655 (abstr.).
10. Horst, K. (1985) Botrytis blight. pp. 18-19. *in: Compendium of Rose Diseases.* The American Phytopathological Society, St. Paul, MN, USA.
11. Jarvis, W.R. (1992) Managing Diseases in Greenhouse Crops. APS Press, St. Paul, MN, USA.
12. Kiraly, Z., Klement, A., Solimosy, F. and Voros, J. (1970) Methods in Plant Pathology. Akadémiai Kiadó, Budapest, Hungary.
13. Köhl, J., Molhoek, W.M.L., Plas, C.H. and Fokkema, N.J. (1995) Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field conditions. *Phytopathology* 85:393-401.
14. Lelliot, M.A. and Stead, D.E. (1987) Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications, London.
15. McKinney, H.H. (1923) Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J. Agric. Res.* 26:195-218.
16. Peng, G. and Sutton, J.C. (1991) Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Can. J. Plant Pathol.* 13:247-257.
17. Pie, K. and Brouwer, Y.J.C.M. (1993) Susceptibility of cut rose flower cultivars to infection by different isolates of *Botrytis cinerea*. *J. Phytopathol.* 137:233-244.
18. Pie, K. and DeLeeuw, G.T.N. (1991) Histopathology of the initial stages of the interaction between rose flowers and *Botrytis cinerea*. *Neth. J. Plant Pathol.* 97:335-344.
19. Redmond, J.C., Marois, J.J. and MacDonald, J.D. (1987) Biological control of *Botrytis cinerea* on roses with epiphytic organisms. *Plant Dis.* 71:799-802.
20. Schaad, N.W. (1989) Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. APS Press, St. Paul, MN, USA.
21. Scott, A.J. and Knott, M. (1974) A cluster analysis method for grouping means in the analysis of variance. *Biometrics* 30:507-512.
22. Sneath, P.H. (1986) Endospore-forming Gram-positive rods and cocci. *in: Sneath, P.H., Mair, N.S., Sharpe, M.E. and Holt, J.G. [Eds.] Bergey's Manual of Systematic Bacteriology.* Vol. 2, pp. 1104-1207. Williams and Wilkins, Baltimore, MD, USA.
23. Sutton, J.C. (1994) Biological control of strawberry diseases. *Adv. Strawberry Res.* 24:1-12.
24. Sutton, J.C. (1995) Evaluation of microorganisms for biocontrol: *Botrytis cinerea* and strawberry, a case study. *in: Andrews, J.H. and Tommerup, I.C. [Eds.] Advances in Plant Pathology.* Vol. II, pp. 173-190. Academic Press, London.
25. Sutton, J.C., Li, D.-W., Peng, G., Yu, H., Zhang, P.G. and Valdebenito-Sanhueza, R.M. (1997) *Gliocladium roseum*: a versatile antagonist of *Botrytis cinerea* in crops. *Plant Dis.* 81:316-328.
26. Sutton, J.C. and Peng, G. (1993) Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Annu. Rev. Phytopathol.* 31:473-493.
27. Sutton, J.C. and Peng, G. (1993) Biocontrol of *Botrytis cinerea* in strawberry leaves. *Phytopathology* 83:615-621.
28. Tatagiba, J. da S. (1996) Avaliação o de microrganismos para o controle biológico de *Botrytis cinerea* em roseira. Dissertação (mestrado). Universidade Federal de Viçosa, Minas Gerais, Brasil.

29. Volpin, H. and Elad, Y. (1991) Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight. *Phytopathology* 81:1390-1394.
30. Washington, W.S., Shanmuganathan, N. and Forbes, C. (1992) Fungicide control of strawberry fruit rots, and the field occurrence of resistance of *Botrytis cinerea* to iprodione, benomyl, and dichlofluanid. *Crop Prot.* 11:355-359.
31. Yu, H. (1996) Relationships of epidemiological factors, *Gliocladium roseum*, and bee vectors to gray mold of raspberry caused by *Botrytis cinerea*. Ph.D. thesis, University of Guelph, Guelph, Canada.
32. Zhang, P.G., Sutton, J.C. and Hopkin, A.A. (1996) Inoculum concentration and time of application of *Gliocladium roseum* in relation to biocontrol of *Botrytis cinerea* in black spruce seedlings. *Can. J. For. Res.* 26:360-367.