Mycologia, 101(2), 2009, pp. 000–000. DOI: 10.3852/08-122

Mycobiota from *Cyclamen persicum* and its interaction with *Botrytis cinerea*

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Abstract: Sixty-six fungi isolated from cyclamen phylloplanes were identified and assessed in vitro for antagonism to *B. cinerea* on leaves, petals, petioles and peduncles. The estimation of pathogen conidial production was used as indicator of biocontrol ability of each of the strains. They were classified by cluster analysis resulting in four categories according to their behavior in the different organs. The most promising category included 34 isolates that significantly reduced pathogen inoculum in all the organs. Correspondence analysis showed association among leaf isolations, strains of *Clonostachys rosea* and *Penicillium* spp. and the best biocontrol performance. The statistical analysis was successful in dealing with this complex set of experimental data. Leaf fungal diversity was higher than those of petals and petiols, with Shannon values of 2.7, 0.9 and 0.5 respectively. Evidence for antibiosis and hyperparasitism was found for *C. rosea*.

Key words: biocontrol, *Clonostachys rosea*, gray mold, phylloplane fungi

INTRODUCTION

Botrytis cinerea Pers.:Fr. is a common and destructive pathogen of pot-grown cyclamen (*Cyclamen persicum* Mill.) in Argentina and has been reported on this host in other countries (Farr et al 1989, Kessel et al 1999). The pathogen causes gray mold, a disease that becomes severe especially when conditions are cool and moist. The main symptoms are necrosis of the leaves and spotting or flecking of the petals. Infected petals that fall onto the leaves can initiate rapid necrosis of major parts of the foliage. Sporulation of *B. cinerea* is frequent on senescing and dead leaves and petals, particularly under moist conditions. Control of gray mold of cyclamen in Argentina presently is dependent on cultural practices and especially fungicide sprays. In recent decades however populations of *B. cinerea* have become resistant to several important fungicides and few alternative chemicals have become available. Furthermore consumers and the public at large are increasingly concerned over fungicide residues on ornamental plants and in the environment (Lange 1992). Biological control is an alternative or complementary plant health measure that is being developed and used against *B. cinerea* in a range of crops (Sutton and Peng 1993b) and might have value for controlling gray mold in cyclamen.

The natural mycobiota associated with living and senescing leaves and flowers of cyclamen is a potential source of microorganisms that are antagonistic to *B. cinerea* and ecologically adapted to growth and activity in association with the host. Selection of effective antagonists from plant-associated mycobiota was successful in several other kinds of plants (Fokkema and der Meulen 1976, Peng and Sutton 1991, Perelló et al 1997, Sutton et al 1997) Nonpathogenic fungi and bacteria, especially those that grow in host tissues at early stages of tissue senescence, often are able to suppress growth and sporulation of *B. cinerea* and other necrotrophs (Heye and Andrews 1983, Clarkson and Lucas 1993, Pfender et al 1993, Sutton et al 1997). Reports on the epiphytic or endophytic mycobiota of cyclamen however are lacking. In the present work we investigated the composition of mycobiota on leaves and flowers of cyclamen plants from commercial greenhouses in Buenos Aires and evaluated fungal isolates for ability to antagonize *B. cinerea* (Rivera 2005).

MATERIALS AND METHODS

Isolation of phylloplane fungi.—Cyclamen plants with no disease symptoms were collected in five commercial greenhouses in Buenos Aires, Jun–Sep 2002, for use as sources of natural fungal flora. One day after collection, 1 cm diam disks were cut from leaf laminae and petals and 2 cm long segments were cut from leaf petioles of 10 plants from each location. Fifty random leaf disks, petal disks and petiole segments were incubated under each of these conditions: (i) in humid chambers (glass slides positioned on humid cotton, in Petri dishes), (ii) on potato dextrose agar medium (PDA), (iii) on nutrient agar medium (NA; Difco) or (iv) on PDA amended with 36 ppm of the herbicide Paraquat. Other groups of 50 tissue pieces were shaken in 250 mL sterile distilled water (SDW) containing 2 drops/L of Triton X-100 surfactant in Erlenmeyer flasks on

Accepted for publication 13 November 2008.
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a rotary shaker (100 rpm). After 2 h the wash water was diluted serially and 0.5 mL aliquots were spread on PDA amended with 100 ppm streptomycin sulphate (PDA) or NA amended with 100 ppm cycloheximide (NA) (Peng and Sutton 1991). In all cases bacterial growth was discarded because the aim of this work was to seek antagonistic fungi.

All plated materials were kept at 21–23 C and were observed daily for microbial growth. Recovered fungi were purified by subculturing on PDA, transferred to PDA slants maintained at 4–8 C to be identified by traditional micromycetes taxonomic keys.

Shannon (Begon et al 2006) diversity index and Sørensen similarity coefficient (Sørensen 1948) were applied to compare fungal biodiversity in the studied organs.

Biocontrol assays.—Each fungal isolate was evaluated for antagonism against *B. cinerea* on aerial organs from cyclamen cv. Nacional Blanco, which is known to be highly susceptible to the pathogen. Each of three replicates per treatment consisted of a humid chamber containing 10 leaf disks, 15 petal disks, four petiole segments or four peduncle pieces. Leaf and petal disks were 1 cm long; petiole and peduncle pieces were 1.6 cm diam × 1.5 cm long. Each chamber with 10 disks or segments was considered an experimental unit.

Groups of organ pieces was washed twice in 200 mL SDW and shaken in an equal volume of SDW at 100 rpm for 1 h to remove surface propagules. The washed organ pieces were placed on glass slides in humid chambers and sprayed with a spore suspension of *B. cinerea* (BAFC Cult 3003) containing 10^6 conidia/mL SDW. After 24 h pieces were sprayed with the various test isolates (10^7 conidia/mL SDW). Inoculum of each isolate was produced by culturing the fungus on PDA at 20 C for 7 d. Density of spores and yeast cells was estimated with a haemacytometer. Controls were sprayed with SDW only. After a further 24 h treated pieces were transferred to paraquat-chloramphenicol agar medium (PCA) in Petri dishes, incubated 7 d at 21 C beneath cool-white fluorescent lights (14 h photoperiod) and examined for sporulation of *B. cinerea*. Paraquat kills plant tissues, thereby allowing *B. cinerea* to sporulate within 7 d. Density of conidiophores of the pathogen on each disk or segment was estimated through a pictorial key (Peng and Sutton 1991) and used as indicator of biocontrol ability of the isolates. The scale consists of eight grades, equivalent to 0, 1–12, 13–24, 25–48, 49–100, 101–200, 201–300 and 301–400 conidiophores per disk. The same scale was applied to petiole and peduncle pieces.

For statistical analyses data recorded on the scale were transformed to median values for number of conidiophores in the various grades. The means of individual medians obtained for each replicate were compared with univariate, one way, analysis of variance and nonparametric analysis of Kruskal Wallis for each organ every time the assumptions of the parametric model were not met. These analyses let us explore the presence of groups of isolates with different control performance. In a second step, because the aim of the study was to find the antagonists with the best global effect for all plant organs, a principal components analysis was carried out with the means of the replicates per isolate for each organ. A matrix with isolates as rows and plant organs where pathogen sporulation was assessed as columns was considered for this analysis to find a linear combination of the assays that could give the best discrimination among isolates. Antagonists subsequently were classified in categories using the first principal component as the variable for grouping. The cluster method was hierarchical using the farthest neighbor technique to construct the tree structures from which clusters could be formed. Statistical computations were performed with Statistical Analysis System (SAS Institute Inc., Cary, North Carolina) and Statistica v.6 (StatSoft Inc., Tulsa, Oklahoma).

TABLE I. Conidiophore density of *B. cinerea* in the bioassays: principal components of total data variation and proportion of variation between isolates

Organ	Principal component			
	First	Second	Third	Fourth
Leaf	0.54	−0.36	−0.27	−0.71
Peduncle	0.46	0.72	−0.48	0.16
Petiole	0.47	0.28	0.83	−0.10
Petal	0.53	−0.52	−0.04	0.67
Eigenvalue	2.52	0.61	0.57	0.30
Percentage variation	63.1	15.2	14.2	7.42

Correspondence analysis was used as an explorative method for the study of associations between the variables: genus (*Clonostachys*, *Penicillium*, *Fusarium*, *Trichoderma*, Other), organ from which the isolate was recovered (leaf, petal, petiole) and category (four levels with decreasing level of control performance).

Interaction mechanisms.—To study interactions between *B. cinerea* and an isolate of the antagonist *Clonostachys rosea*, the two fungi were grown in dual cultures on PDA in Petri dishes or on films of PDA on glass slides positioned in humid chambers. The fungi were examined after 4–6 d incubation at 20 C by means of light microscopy (Zeiss Axioscop) and environmental scanning electron microscopy (Philips Electroscan 2010). Specimens to be observed were prepared by cutting 1 cm^2 agar blocks with mycelia.

RESULTS

Isolation of phylloplane fungi.—Sixty-six fungal isolates were obtained and identified to genus or species (TABLE II). The fungi included *Penicillium* spp. (26), *Clonostachys rosea* (10), *Trichoderma* spp. (10), *Fusarium* spp. (7), *Acremonium* spp. (4), yeasts (3), *Phoma* sp. (2), *Aspergillus* spp. (2), *Chaetomium* (1) and an unidentified Hyphomycete (1). *B. cinerea* was encountered in all cyclamen samples and in most treatments.

Fungal diversity was greater in leaves, with 20 species, compared with petals and petioles (eight species each), respectively showing Shannon values of 2.7, 0.9 and 0.5. Regarding similarity among organ

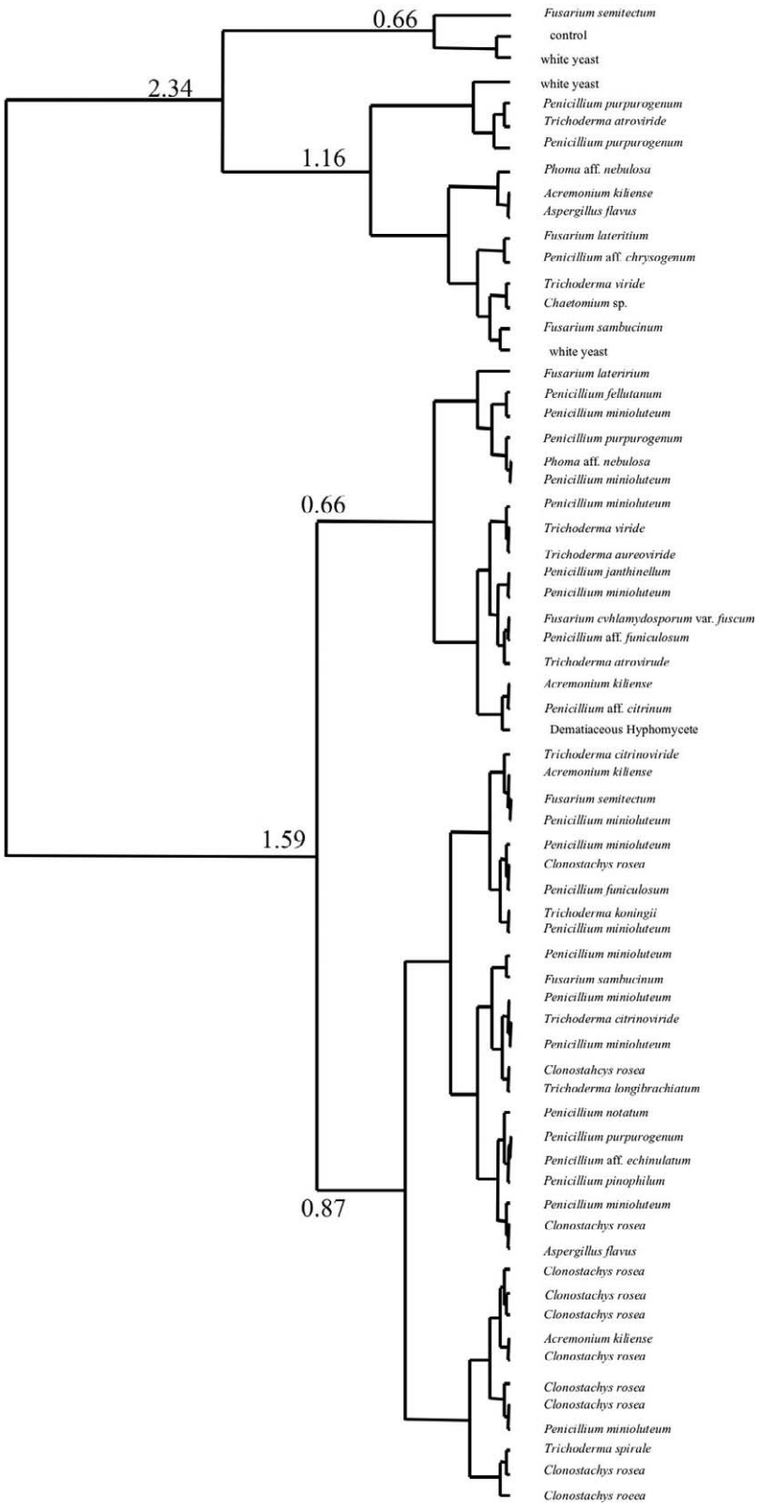

FIG. 1. Cladogram of phylloplane species obtained from cyclamen crops according to their antagonism against *B. cinerea*.

TABLE II. Isolates classified according to their antagonism against *Botrytis cinerea* on different cyclamen organs

Isolate	Genus and species	Isolation Organ	First Compo-nent	Mean conidiophore density of *B. cinerea* on cyclamen organs			
				L	Pd	Pt	Pl
		Category 1					
57	*Acremonium kiliense*	Pl	−0.7	125	18	0	29
37	*Acremonium kiliense*	Pt	−1.6	30	0	0	15
2	*Aspergillus flavus*	L	−1.3	21	56	5	38
43	*Clonostachys rosea*	L	−1.3	63	0	28	10
55	*Clonostachys rosea*	L	−1.0	44	94	39	16
40	*Clonostachys rosea*	L	−0.6	73	97	63	23
8	*Clonostachys rosea*	L	−1.6	2	80	6	9
47	*Clonostachys rosea*	L	−1.6	22	46	0	2
6	*Clonostachys rosea*	L	−1.9	2	0	0	19
67	*Clonostachys rosea*	L	−1.7	31	0	15	0
64	*Clonostachys rosea*	L	−1.5	2	125	0	1
10	*Clonostachys rosea*	Pt	−1.5	20	51	0	11
1	*Clonostachys rosea*	Pt	−2.0	4	0	0	0
53	*Fusarium semitectum*	L	−0.7	51	36	36	77
12	*Fusarium sambucinum*	Pl	−1.1	67	9	9	36
31	*Penicillium* aff. *echinulatum*	L	−1.2	46	94	0	0
18	*Penicillium minioluteum*	L	−0.7	119	46	23	5
4	*Penicillium minioluteum*	L	−1.4	49	14	2	16
17	*Penicillium minioluteum*	L	−1.4	39	21	21	14
15	*Penicillium minioluteum*	L	−1.1	84	38	14	5
3	*Penicillium minioluteum*	L	−1.3	24	46	9	33
9	*Penicillium minioluteum*	Pt	−0.5	63	75	75	46
32	*Penicillium minioluteum*	Pt	−0.9	88	0	81	19
38	*Penicillium minioluteum*	Pt	−0.6	95	9	28	59
11	*Penicillium minioluteum*	Pl	−0.9	73	36	36	28
25	*Penicillium funiculosum*	Pt	−0.6	60	63	127	15
7	*Penicillium notatum*	L	−1.2	43	56	56	0
5	*Penicillium pinophilum*	L	−1.3	49	37	18	15
66	*Penicillium purpurogenum*	L	−1.2	80	0	23	0
19	*Trichoderma citrinoviride*	L	−0.9	66	27	27	45
20	*Trichoderma citrinoviride*	L	−0.8	61	80	27	39
21	*Trichoderma koningii*	L	−0.5	56	113	37	58
11	*Trichoderma longibrachiatum*	Pt	−1.0	37	51	23	58
54	*Trichoderma spirale*	L	−1.9	10	0	0	41
		Category 2					
57	*Acremonium kiliense*	Pl	−0.3	80	226	0	22
13	Dematiaceous Hyphomycete	Pl	−0.4	108	37	18	72
50	*Fusarium chlamydosporum* var. *fuscum*	L	−0.1	109	150	9	52
56	*Fusarium lateritium*	L	0.7	74	213	218	39
41	*Penicillium* aff. *citrinum*	L	−0.2	5	163	63	105
24	*Penicillium* aff. *funiculosum*	Pl	−0.1	109	18	9	37
61	*Penicillium fellutanum*	L	0.5	137	326	10	1
34	*Penicillium janthinellum*	Pt	−0.1	58	301	38	0
23	*Penicillium minioluteum*	Pt	0.2	119	67	103	75
36	*Penicillium minioluteum*	Pt	0.4	121	207	85	35
35	*Penicillium minioluteum*	Pt	0.1	88	111	201	7
30	*Penicillium minioluteum*	Pl	−0.2	55	301	38	0
45	*Penicillium purpurogenum*	L	0.3	70	144	119	82
48	*Phoma* aff. *nebulosa*	L	0.3	61	0	351	28
16	*Trichoderma atroviride*	L	−0.0	164	0	42	65
28	*Trichoderma aureoviride*	Pl	0.1	93	65	23	127
60	*Trichoderma viride*	Pl	0.0	130	0	0	134

TABLE II. Continued

Isolate	Genus and species	Isolation Organ	First Compo-nent	Mean conidiophore density of *B. cinerea* on cyclamen organs			
				L	Pd	Pt	Pl
		Category 3					
39	*Acremonium kiliense*	Pt	1.1	81	207	138	143
29	*Aspergillus flavus*	Pt	1.2	54	151	151	199
33	*Chaetomium* sp.	Pl	1.9	201	301	125	64
52	*Fusarium lateritium*	L	1.4	111	351	125	77
46	*Fusarium sambucinum*	L	1.7	36	351	282	110
42	*Penicillium* aff. *chrysogenum*	L	1.5	215	276	38	66
62	*Penicillium purpurogenum*	L	2.7	137	351	351	90
44	*Penicillium purpurogenum*	L	2.9	222	213	282	142
49	*Phoma* aff. *nebulosa*	L	1.0	79	0	351	114
51	*Trichoderma atroviride*	L	2.8	188	276	301	115
59	*Trichoderma viride*	Pl	1.9	169	276	14	184
22	White Yeast	L	1.6	210	51	51	204
65	White Yeast	L	2.4	175	251	0	271
		Category 4					
68	Control	–	5.0	280	346	346	246
63	*F. semitectum*	L	3.9	246	150	326	246
27	White Yeast	Pt	4.8	260	351	276	282

[L] leaf; [Pd] peduncle; [Pt] petiole; [Pl] petal

mycobiota, Sørensen indexes were 0.34 between petals and petioles, 0.29 between leaves and petioles and 0.14 between leaves and petals.

Biocontrol assays.—Observations of effects of the isolates on conidiophore density of *B. cinerea* in the bioassays were used for global analysis. Although assumptions for the parametric analysis of variance were not met, results with Kruskal Wallis tests were similar for all organs, showing significant differences among means of conidiophore density, with H Statistic of 154.34, 153.74, 160.23 and 161.89 for leaf, petal, petiole and peduncle respectively, all with p-values < 0.0001. Once the differences among means of conidiophore density for all organs were found, a principal component analysis provided the three principal components that explained about 93% of data variation (TABLE I). The first component explained 63% of total variation; all coefficients were positive and of similar magnitude. The second principal component seemed to represent a contrast between conidiophore density of leaf, petal and peduncle, petiole. The third principal component that explained about 14% of the variability seemed to measure the difference between petiole and the rest of the plant organs. We selected only the first principal component for cluster analysis because it was the only one with an eigenvalue greater than 0.7 (Jolliffe 1972) and for its usefulness in evaluating the isolates global performance. Low values of this component (low pathogen conidiophore density) indicate high ability of the isolates to antagonize the pathogen.

Four categories of isolates that were homogeneous with respect to antagonism of *B. cinerea* emerged from the global analysis and comprised respectively 34, 17, 13 and two isolates (FIG. 1). Isolates in any categories did not completely suppress conidiophore production by *B. cinerea* in at least one of the cyclamen organs tested. Relative suppression of *B. cinerea* by isolates in Categories 1, 2 and 3 respectively were high, intermediate and low (TABLE II). However some isolates in Category 3 totally suppressed conidiophore production in some cyclamen organs used but had little effect in other organs. Category 4 included two isolates that failed to suppress *B. cinerea*.

The most effective isolates (Category 1) were mainly *C. rosea* and species of *Penicillium* and *Trichoderma*. Isolates of some species of *Penicillium* (e.g. *P. minioluteum*) also performed at a lower level of efficacy and were assigned to Category 2. However all isolates of *C. rosea* fell into Category 1 and among these isolate 1 was the most suppressive to *B. cinerea*. This isolate was recovered from a petiole of cyclamen and completely inhibited sporulation of *B. cinerea* in all organ tissues tested except leaf laminae on which a few conidiophores of the pathogen developed. The correspondence analysis let us explore the relationship among genera, isolation organs and biocontrol categories. The 33.7% of the total inertia was

TABLE III. Correspondence analysis of genus, isolation organ and biocontrol category

	Dimension 1	Dimension 2	Quality
Genus: Other	−1.1	0.6	0.4
Genus: *Penicillium*	0.1	−0.7	0.3
Genus: *Clonostachys*	1.6	0.3	0.5
Genus: *Trichoderma*	−0.3	−0.4	0.0
Genus: *Fusarium*	−0.4	1.6	0.3
Organ: Leaf	0.3	0.3	0.4
Organ: Petiole	0.0	−0.4	0.0
Organ: Petal	−1.3	−0.7	0.4
Category: 1	0.8	−0.1	0.6
Category: 2	−0.8	−0.9	0.5
Category: 3	−0.8	1.0	0.4
Category: 4	−0.8	2.8	0.3
Eigen Value	0.5	0.5	
Percent Inertia	18.4	15.3	
Cumulative Percent	18.4	33.7	

explained by the first two dimensions (TABLE III, FIG. 2). It appears that the first dimension distinguishes mostly between Category 1 and the rest of the categories and that this first category is associated with leaf and genera *Clonostachys* and *Penicillium*, indicating that these genera and organ are associated with the best control performance. The second dimension separates Category 2 from the categories with the worst performance, and these categories appear to be associated with genus *Fusarium* and the genera considered in the ''other'' class. Furthermore the third quadrant of the plot indicates that the Category 2 is associated with petal and *Trichoderma*.

Interaction mechanisms.—Antibiosis, evidenced by zones of inhibition of the pathogen mycelial growth,

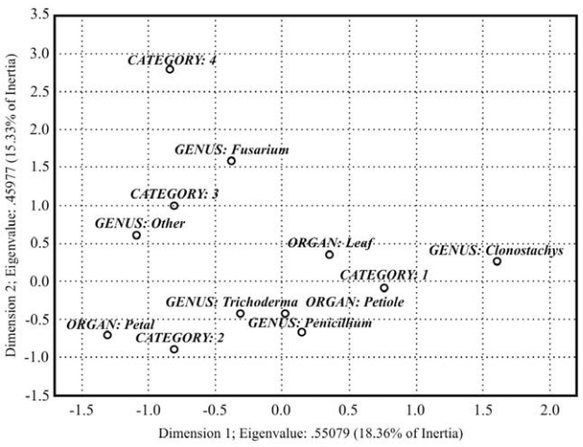

FIG. 2. Correspondence analysis of associations between isolate genus, organ from which they were recovered and biocontrol category.

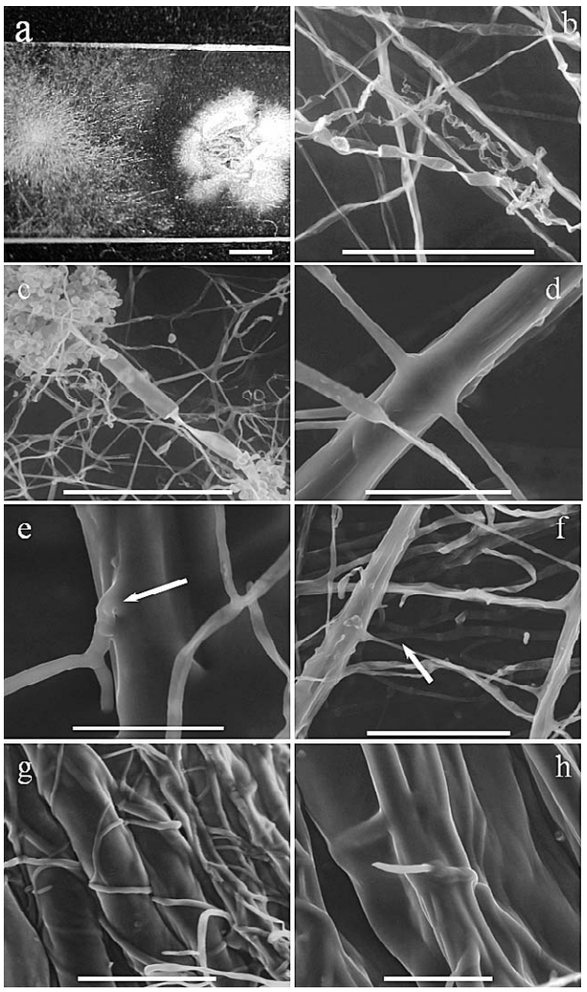

FIG. 3. Interactions between *B. cinerea* and *C. rosea* in 5–6 d dual cultures. a. Zone of inhibition. b. *B. cinerea* mycelium showing ribbon-like, twisted, empty and collapsed hyphae. c. *B. cinerea* plasmolized conidiophore cells. d. *C. rosea* hyphae growing perpendicularly toward *B. cinerea*. e. *C. rosea* appressoria over *B. cinerea* (arrow). f. *Clonostachys* hyphae linking pathogen hyphae (arrow). g. *C. rosea* hyphae coiling around *B. cinerea*. h. *C. rosea* emerging from hyphae of *B. cinerea*. Bars a = 2 cm; b, g = 100 μm; c = 150 μm; d = 25 μm; e = 35 μm; f, h = 50 μm.

was observed in dual cultures of *C. rosea* and *B. cinerea* (FIG. 3a). Pathogen hyphae showed morphological alterations in the interaction area as well as distant areas from *C. rosea*, suggesting activity of metabolites either volatile or highly diffusible in the agar media. Mycelium and conidiophores of *B. cinerea* were ribbon-like, twisted, empty and collapsed (FIG. 3b). Plasmolized cells were observed between apparently normal ones in the pathogen conidiophores (FIG. 3c). *C. rosea* shows lineal growth, perpendicular toward hyphae of *B. cinerea*, in many

observations (FIG. 3d). The antagonist grew abundantly over the pathogen hyphae, developing appressoria on them (FIG. 3e). *Clonostachys* hyphae linked (FIG. 3f) and coiled *B. cinerea* (FIG. 3g), sometimes emerging from the latter (FIG.3h).

DISCUSSION

As far as we know this is the first time a principal component analysis was applied to sporulation grades resulting from the interaction between a pathogen and phylloplane organisms to rank their potential effectiveness and build biocontrol categories. A further correspondence analysis let us define factors related to the grouping. In our case the factors were the organism genus and the isolation organ. In this way each isolate could be evaluated through its behavior on the different organs of the plant.

Mycobiota from cyclamen phylloplanes in commercial greenhouses in Buenos Aires exhibited a major diversity on leaves, suggesting that this could be the best source for selecting native biocontrol agents against gray mold. Whether it depends on differential availability of nutrients in host exudates remains to be studied. It is generally accepted that the biocontrol effectiveness depends more on strain than on species, which was supported in our results.

Based on the methods used to recover the isolates, the fungi probably were present on the phylloplane or potentially within the tissues as nonpathogenic endophytes. While the tissues used were asymptomatic, this did not rule out the possibility of isolates being pathogenic to cyclamen, as was the case with *B. cinerea*. Moreover absence of records in the literature suggests that none of the isolated species except *B. cinerea* are pathogenic to cyclamen, a characteristic of obvious importance in selecting antagonists against the pathogen. But some of them, despite their antagonistic ability, are not suitable for biofungicide development due to the production of toxic metabolites; these are *Fusarium lateritium*, *F. sambucinum*, *F. semitectum* and *F. chlamydosporum* (Nelson et al 1983). Other species should not be considered because of their potential pathogenicity. For example *Penicillium minioluteum*, the most frequently isolated species, has been reported as causal agent of bulb rot on tulip (*Tulipa* sp.), gladiolus (*Gladiolus* sp.) and lily (*Lilium multiflorum*) in different locations of Argentina (Wright et al 2003).

Similarity coefficients did not resemble the organs relative position in the plant because for example the higher values were obtained between petals and petioles, which are distant from each other. In spite of the higher diversity on leaves, the best performance strain was isolated from petal, which showed low diversity and the lowest similarity coefficients with leaves.

C. rosea has emerged as an effective and versatile antagonist of *B. cinerea* that would improve crop health management (Sutton et al 1997). It has been reported for example as antagonist of *Phytophthora megasperma* f. sp. *glicinea* (Al-Heeti and Sinclair 1988), *Verticillium dahliae* (Keinath et al 1991), *Septoria tritici* (Perelló et al 1997) and *Monilinia fruticola* (Wittig et al 1997). As biocontrol agent of *B. cinerea*, it has been studied on strawberry (Peng and Sutton 1991; Sutton and Peng 1993a, 1993b), begonia (Li et al 1995), black spruce (Zhang et al 1996), rose (Boechat Morandi 1997, Silva Tatagiba et al 1998), bean (Szandala and Backhouse 2001) and tomato (Shafia et al 2003). Li et al (1996) and Köhl et al (1998) controlled *B. cinerea* by spraying conidial suspensions of *C. rosea* on cyclamen.

C. rosea acts by different mechanisms (Soares de Melo, 1996), such as competition for nutrients (Sutton and Peng 1993b) and for substrate (Sutton and Peng 1993a) and mycoparasitism (Pachenari and Dix 1980, Yu and Sutton 1994). In this work mechanisms of antibiosis and mycoparasitism were demonstrated in the interaction between *C. rosea* and *B. cinerea*. Antibiosis was documented by the observation of inhibition zones between colonies and collapsed pathogen hyphae and conidiophores. The presence of the antagonist appressoria and hyphae growing toward the pathogen and invading it constitute evidences of mycoparasitism. We suggest that both mechanisms are involved in this fungal interaction.

According to Köhl et al (1995) a biocontrol strategy based on competition may decrease the growth and sporulation of the pathogen on dead tissues. As a consequence reduction in the sporulation of *Botrytis* spp. may result in a delay of epidemics. An additional advantage of this strategy is the long-term interaction between pathogen and antagonist in crop residues. In our work most of cyclamen phylloplane fungi are able to reduce pathogen sporulation. These results should be confirmed in the field.

The preselection on plant tissues in vitro (Peng and Sutton 1991) may help to reduce frequent inconsistencies between results in lab and field conditions (Elad 1990, Soares de Melo 1996). Besides local isolates may increase biocontrol success because of their adaptation to the host, environmental and crop conditions. Further research should be focused on testing mixtures with other antagonistic phylloplane microorganisms, complementary greenhouse management measures and also low toxicity fungicides. Research on space-time composition of cyclamen fungal communities would be useful to understand

naturally occurring biological control and the effect of crop management on them.

ACKNOWLEDGMENTS

We gratefully thank Dr John C. Sutton (University of Guelph) who kindly reviewed the manuscript and improved the English. This work was financially supported by the University of Buenos Aires.

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