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Endophytic Fungi from Uruguayan Native Myrtaceae: Enzymes Production, Antimicrobial and Phytotoxic Activity

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Abstract: In recent years fungal endophytes occurring in native Myrtaceae from Uruguay have been studied. Fungal associations with the host, such as saprotrophs, latent plant pathogens or symbiotic, are related with enzymes and secondary metabolites production. Therefore, a main goal of this work was to evaluate the ability of endophytic fungi isolated from *Myrcianthes cisplatensis*, *Myrrhinium atropurpureum* and *Eugenia uruguayensis* to produce hydrolytic and oxidative extracellular enzymes and bioactive metabolites with antimicrobial activity or phytotoxic properties. Enzymes production were evaluated by plate assay and metabolites were extracted with organic solvents. For detecting phytotoxicity, fungal extract were injected on fresh leaves of *E. uruguayensis*. Antimicrobial activity was evaluated by antibiogram technique and growth inhibition assessed by halo. *Cladosporium sphaerospermum*, *Xylaria acuta* and Amphisphariaceae 143 that showed the highest enzymatic activity probably degrading plant debris when they arrive to soil having saprotrophic life style. The enthomopathogenic fungus *Metarhizium anisopliae* producing proteases and chitinases is active pathogen of several insect species. In addition, *Nigrospora spherica* and *Xylaria* sp. produced necrotic spots on Myrtaceae leaves evidencing phytotoxic activity. The antifungal activity showed by some endophytic species evidenced the ability to limit the development of microorganism populations. *Lophiostoma* sp. 246 was the most active strain against *Staphyloccocus aureus* and *Candida albicans* and *Preussia. africana* evidenced a good antimicrobial activity against *Xanthomonas campestris.* Native Myrtaceae seems to be a good source of fungal endophytes for producing enzymes related with decomposition process and a promising source of bioactive metabolites with antimicrobial and fitotoxigenic activities. In addition, the new strain of *M. anisopliaes* could be a promising bioinsecticide.

Keywords: Secondary metabolites, endocellulases, pectinases, ligninases, proteases, chitinases

Introduction

In recent years fungal endophytes occurring in native Myrtaceae from Uruguay have been studied (Bettucci et al. 2004; Tiscornia et al. 2012). Several genera of this large family of plants are distributed in tropical and subtropical forests in south-eastern of South America (Brazil, Uruguay, northeast Argentina, south-central Paraguay), mainly between 20 - 35 ° S and 48 - 56° W, with a few genera restricted to the Andean highlands of the northwest (Brussa and Grela 2007). As the function of endophytes in woody plants is less clear than in grasses, different visions have characterized them establishing associations with the host such as saprotrophs, latent plant pathogens or symbiotics, but in few cases relations were clearly evidenced (Saikkonen 2007; Sieber 2007; Promputtha et al. 2010).

The growth of most endophytes depends on readily available compounds as soluble sugars. The ability of endophytic fungi to produce enzymes for degrading cellulose and lignin is a probable strategy that allows some endophytes to decay tissues and persist as saprobes after host senescence. Extracellular enzymes produced by several species under appropriated conditions revealed, at least, some important function as decomposer in the ecosystem (Oses et al. 2006). Moreover, it is known that 60% of enzymes used in industrial processes are produced by few genera of fungi ubiquitous and of world wide distribution (Suryanarayanan et al. 2012).

Secondary metabolites produced by several endophytic fungal species can display antimicrobial or phyotoxical effect. Phytotoxins are chemicals of low molecular weight that can produce death or distort the host cells acting at very low concentration (Aly et al. 2010). Mutant of pathogenic strains becoming unable to produce toxins can lose their virulence (Beresteskiy 2008). Endophytic species with antimicrobial properties activate host defences more quickly than non symbiotic plants when exposed to virulent pathogens (Lv et al. 2010).

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In agreement with these facts we focus here to evaluate fungal endophytes from native Myrtaceae as enzymes producers evidencing a sprotrophic life style, and as potential pathogens producing phytotoxins. In addition antimicrobial activity showed by some species could evidence the ability to produce extracellular metabolites for regulation of microorganism populations. These species which produce antimicrobial metabolites could also be used against human and plant pathogens bacteria and fungi.

Therefore, a main goal of this work was to evaluate the ability of endophytic fungi isolated from *Myrcianthes cisplatensis*, *Myrrhinium atropurpureum* and *Eugenia uruguayensis* to produce hydrolytic and oxidative extracellular enzymes and bioactive metabolites with antimicrobial activity or phytotoxic properties.

Materials and methods

Extracellular enzymes produced by several species under appropriated conditions could reveal, at least, some important function of endophytes as decomposers or phytopathogens.

Enzymes production

Endocellulases. They were determined using carboxy methyl cellulose (CMC) into a basal medium (Eggins and Pugh 1962). CMC allow evidencing the endocellulases production. A fungal isolate from each species was inoculated and three replicate were performed. Enzymes production was revealed adding 0.25% Congo Red on the surface of culture medium. The excess of colorant was discarded and the surface was rinsed with 0.5 M of NaCl. The enzymatic activity was visualized as a clearance halo around the colonies. The diameter of mycelial colony (Dm) and that of the clearance halo (Ch) were measured. The enzymatic activity (Ea) was calculated as Ch/Dm. If the result was higher than 1 it means that the enzyme production diffuses outside the mycelium of advancing zone. As it is a semi quantitative method as higher is the relation more is the enzymatic activity.

Pectinases. A basal medium with citric pectin as a unique carbon source to detect pectinases activity was used. A fungal isolate from each species was inoculated and three replicate were performed. Plates were flooded with 0.1 Ruthenium red solution incubated during one hour (Oliveira et al. 2006). Then, the Ruthenium red solution was removed from the plates and the surface of the medium was rinsed with distilled water. The pectin clearance halo (Ch) was measured. The enzymatic activity was calculated as for endocellulases.

Ligninases. The ligninases activity was evaluated using as substrate Remazol Brilliant Blue R (RBBR). A fungal isolate of each species was inoculated on Malt–Agar 2% containing 0.05 % RBBR (Machado et al. 2005). Clearance areas of the culture medium around the colony indicated enzyme activity after 15 days of incubation at 25 ºC. Discoloration indicating enzyme activity was evaluated as negative (-), light (+) and positive reaction increasing from (++) to (++++).

Extracellular enzymes from entomopathogenic fungi

These enzymes are produced by isolates belonging to species known as entomopathogens. They are related to the virulence of entomopathogenic fungi, allowing the hyphae penetrate the cuticle and colonize tissues in the insect host. Enzymes production were determined by digestion of the substrate incorporated (either dissolved or suspended) in solid culture media.

Entomopathogenic fungus was inoculated in Petri dishes containing the media with substrates for different enzymes and incubated for 7 to 14 days at 25 ° C. The clearance halo around colonies reflecting the enzyme activity was measured in the the respective media as the diameter of colony. Enzyme activity (EA) was calculated as the ratio of clearance diameter (HD) and mycelial growth diameter (MD).

Proteases. For determination of protease 3 discs of 7 mm in diameter containing mycelium and spores of the entomopathogenic fungus were introduced in Petri dishes containing agar milk casein (Varela 1998). The production of proteases was shown as cleared areas around the colonies indicating the hydrolysis of the substrate. The diameter of colony and the clearance halo were measured.

*Chitinases.*For determination of chitinases production the fungal isolate was inoculated as was described above but in Petri dishes containing salts medium according Chul-Kang et al. (1999) supplemented with colloidal chitin as substrate (Mier et al. 2004). The diameter of colony and the clearance halo were measured.

Secondary metabolites

Secondary metabolites produced by several endophytic species can display antimicrobial or phyotoxical effect.

Antimicrobial activity. The extraction of metabolites was performed from pure cultures on malt agar. The agar from each colony was sectioned in grids with sterile scalpel, placed in vials and metabolites were extracted with ethyl acetate (Smedsgaard 1997). Antimicrobial activity from each extract obtained was evaluated against bacteria *Escherichia coli* (Migula) Castellani and Chalmersvon, *Staphylococcus aureus* Rosenbach, *Erwinia carotovora* (Jones) Bergey et al. and *Xanthomonas campestres* (Pammel) Dowson, and against

pathogenic fungi *Aspergillus fumigatus* Fresen., *Candida albicans* (C.P. Robin) Berkout, *Colletotrichum gloeosporioides* (Penz) and *Fusarium oxysporum* (Schlecht.). Antibiogram technique was applied and growth inhibition assessed by halo formation (Bauer et al. 1966).

Phytotoxic activity
Metabolite was tested on *Eugenia* leaves, in damp chambers. On the adaxial surface of each leaf, three or four needle puncture wounds were made on each side of the mid vein. In wounds on one side 10 µl of metabolite ⁄ wound was injected and in wounds on the other side acetone-only was injected (controls). At least five replicate leaves were assayed for each extract, in 12 h dark: 12 h light with 72 W black light and 36 W white light tubes at 22ºC and monitored for up to16 days.

Results
Enzyme activity
A high percentage (98%) of endophytic isolates produced a clarence halo in the culture media used for the determination of any extracellular enzymes.

Table 1 shows the species with cellulolytic, pectinolytic and lignolytic activity. From 103 isolates, 23 (22.33%) exhibited positive activity for the three enzymes evaluated, *Lophiostoma* sp., *C. sphaerospermum*, *X. acuta* and the isolate Xylariales 143 showed the highest performance.

Endocellulases: 20 isolates (19.4%) evidenced activity equal or higher than 1.5. The species with highest activity were *Aureobasidium pullulans* (de Bary) G. Arnaud (3.22), *Paraconyothirium fungicola* (2. 92), *Xylaria* sp., (2.52) and *Bartalinia robilliarioides* Tassi (2.29).

Pectinases: 13 isolates (12.6%) evidenced an activity over 1.5, being the isolates Xylariales 143 (3.29), Xylariales 101 (3.13), *Paecilomyces variotii* (2.03) and *Xylaria acuta* (1.85) the highest producers.

Ligninases: 21 isolates (20.38 %) showed a high positive activity evaluated by RBBR dye discoloration being *Lentinus trigrinus* (Bull.) Fr., *Neofusiccocum autrale*, *Nodulisporium* sp., *Phlebiopsis gigantea* and *Xylaria* sp. those with the highest activity.

The endophytic entomopathogen isolated from twig xylem produced proteinases and chitinases.

Proteinases. Metarhizium anisopliae 238 showed the highest enzymatic activity at the second day of fungal inoculation.

Chitinases. Conversely, this fungus did not evidence enzymatic activity at the second day showing the highest enzyme production at 14 th day (Table 2).

Antimicrobial activity. From 23 isolates evaluated for their secondary metabolites production, 19 exhibited antimicrobial activity at least on one tested microorganism. One isolate of *Lophiostoma* sp. 246 was active vis à vis all tested organisms particularly on *Staphyloccocus aureus* and *Candida albicans.* Other isolates with some activity on *S. aureus* were *Alternaria alternata* and *Sclerostagonospora opuntiae. Preussia africana* evidenced a good antimicrobial activity to *Xanthomonas campestris* (Table 3).

Phytotoxicity . From the 29 endophytes tested, 9 isolates produced metabolites that evidenced some fitoxytoxic activity on leaves of *E. cisplatensis. Nigrospora spherica* isolated from petiole and *Xylaria* sp. isolated from blade of *Myrciantes cisplatensis* produced necrotic spots on the inoulated area, after 16 days (Table 4).

Discussion
There are several reports on the role of endophytes as causing chemical changes in decomposing process suggesting that their mode of life can change to saprobes on leaf litter (Shirouzu et al. 2009). A high percentage (98%) of endophytes isolated here were able to produce any of cell wall decomposing enzymes, endocellulases, pectinases and ligninanses. The white rot *L. tigrinus* and *Ph. gigantea*, the soft rot *Nodulisporium* sp. and *Xylaria* sp. can contribute to degradation of lignin, cellulose and pectin components of plant debris in soil and litter (Boddy and Griffith and 1989; Pointing et al. 2003; Oses et al. 2006). In agreement with Petrini et al. (1995) and Whalley (1996) the role of endophytic *Xylaria* species is their tendency to be dormant until host senescence at which time they start the decomposition of host tissues reflecting the saprophytic ability of these species. This provides them with the advantages over other competing saprotrophs in the uses of available nutrient resources from host tissues (Davis et al. 2003; Promputha et al. 2006). *L. tigrinus* has been successfully selected for lignin degradation of sugar cane bagasse (Breccia el al. 1997) and other lignocellulosic materials (Bettucci et al. 1998). Moreover, *L. tigrinus* is capable in removing organic load, colour and toxic phenols from agro-industrial effluent olive-mill wastewater (Fenice et al. 2005).

Proteinases and chitinases produced by *M. anisopliae* are related to the virulence of this entomopathogenic fungus, allowing the hyphae penetrate the cuticle and colonize tissues in the insect host, where it produces toxins that kill the cells. After, the fungus changes the mode of growth and emerges from the insect host producing spores. This *Metarhizium* isolate has a

high entomopathogenic ability under laboratory conditions (Tiscornia 2012).

Endophytic fungi have open a good source in the search of metabolically active compounds from native plants from South America neotropical forests that have been relatively unexplored as fungal hosts (Hormazabal and Piontelli 2009). According with Dreyfuss and Chapela (1994) about 4000 secondary metabolites of fungal origin have been described as biologically active. The inhibition activity of fungal extracts could be considered a promising source of new antibiotics. Conversely, *N. sphaerica*, and *Xylaria* sp.120 were the main phytotoxic extract producers. This fact suggests that the latent pathogenic ability of some endophytic fungi remains to be known. Other intriguing problem is if phytotoxic metabolites are produced inside plant tissues by the endophytic fungi in enough amounts to produce the same interaction.

Native Myrtaceae plants seems to be a good source of endophytes producing enzymes related with decoposition process and bioactive metabolites with antimicrobial and fitotoxigenic activities. In addition, the presence of the entomopathogenic fungus *M. anisopliaes* a bioinsecticide for biocontrol are a novel source of organisms to be study for biotechnological selection.

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Table 1. Production of extracellular enzymes: endocellulases, pectinases and ligninases

Endophytic isolates	Endocellulases EA	Pectinases EA	Ligninases Clearing
Alternaria alternata (Fr.) Keissl. (101)	1,17	1,05	-
Alternaria alternata (Fr.) Keissl. (5)	1,06	1,12	++
Anthostomella sp. Sacc. (25)	1,22	-	-
Ascomycota (146)	-	1,82	++
Ascomycota (150)	1	-	-
Aureobasidium pullulans (de Bary) G. Arnaud. (8)	3,22	1,41	-
Bartalinia robillardoides Tassi (236)	2,29	-	-
Beltrania rombica Penz. (218)	1	1,2	++
Cladosporium cladosporioides (Fresen) de Vries (239)	1,47	1,67	-
Cladosporium cladosporioides (Fresen) de Vries (48)	2,6	1	-
Cladosporium sphaerospermum Penz. (240)	1,86	1,3	+++
Colletotrichum acutatum J.H. Simmonds (50)	1,66	1,49	-
Colletotrichum gloeosporioides (Penz) Penz & Sacc. (1')	1,13	1,15	-
Colletotrichum gloesporioides (Penz) Penz & Sacc. (205)	1,19	1,17	-
Coniochaeta velutina (Fuckel) Cooke (221)	-	-	-
Conoplea fusca Pers. (247)	1,08	-	-
Corynespora sp. Güssow (53)	-	2	++
Daldinia sp. Ces. & De Not. (229)	1,48	-	-
Diaporthe sp. Nitschke (224)	1,25	-	+
Epicoccum purpurascens Ehrenb. (45)	1,06	-	-
Eupenicillium brefeldianum (B.O. Dodge) Stolk & D.B. Scott (211')	1,34	1,54	++
Gelasinospora retispora Cain (38)	1	1	+
Glomerella cingulata (Stoneman) Spauld & Schrenk (223)	1	1,14	+
Guignardia mangiferae A. J. Roy (116)	-	-	+++
Lecythophora fasciculata (J.F.H. Beyma) E. Weber, Görke & Begerow (55)	1,2	-	++
Lecythophora fasciculata (J.F.H. Beyma) E. Weber, Görke & Begerow (121)	1,33	1,54	++
Lentinus tigrinus (Bull.) Fr. (207)	0,88	-	++++
Lophiostoma Ces. & De Not. (246)	1,91	1,25	+++
Lophiostoma Ces. & De Not. (57)	1	-	++
Metarhizium anisopliae (Metschn.) Sorokīn (238)	-	-	-
Microdiplodia hawaiiensis Crous (56)	1,69	1,26	-
Morinia pestalozzoides Berl. & Bres. (34)	1,85	-	+++
Myrothecium verrucaria (Alb. & Schwein.) Ditmar (152)	1,42	-	-
Neofusicoccum australe (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips (132)	0,68	1,07	++++
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips (12)	-	-	+
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips (205')	-	1	+
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips (212)	0,67	1	-
Nigrospora sacchari (Speg) Mason (201)	0,89	-	-
Nigrospora sphaerica (Sacc) Mason (113)	0,78	1,12	-
Nigrospora sphaerica (Sacc) Mason (216)	0,83	-	-
Nigrospora sphaerica (Sacc) Mason (4)	1,08	-	-
Nodulisporium sp. Preuss (125)	1,35	-	++++
Nodulisporium sp. Preuss (18)	1	-	+
Paecilomyces variotti Bainier (51)	2,02	2,03	-
Paraconiothyrium fungicola Verkley & Wicklow (119)	1,18	1,63	++++

Paraconiothyrium fungicola Verkley & Wicklow (142)	2,92	1	++
Pestalotiopsis guepinii (Desm) Steyaert (104)	0,91	1,09	+++
Pestalotiopsis guepinii (Desm) Steyaert (231)	1,01	1,14	-
Peyronellaea australis Aveskamp, Gruyter & Verkley (128)	-	1	+
Peziza varia (Hedw.) Fr. (58)	0,92	-	-
Phaeoacremonium sp. W. Gams, Crous & M.J. Wingf. (20)	1,26	1	+
Phlebiopsis gigantea (Fr.) Jülich (159)	1	1	++++
Phoma glomerata (Corda) Wollenw. & Hochapfel (13)	0,98	1,04	+
Phomopsis sp.(Sacc) Bubák (202)	-	1,08	-
Phomopsis sp.(Sacc) Bubák (204)	1	1,12	-
Phomopsis sp.(Sacc) Bubák (206)	1,08	1,6	-
Phomopsis sp.(Sacc) Bubák (213)	-	1,07	-
Phomopsis sp.(Sacc) Bubák (123)	1,33	1,3	-
Phomopsis sp.(Sacc) Bubák (203)	1	1,31	-
Phomopsis sp.(Sacc) Bubák (21)	1,72	-	++
Phomopsis sp.(Sacc) Bubák (214)	0,79	1,18	-
Phomopsis sp.(Sacc) Bubák (217)	-	1,31	-
Phomopsis sp.(Sacc) Bubák (219)	0,69	1,33	-
Phomopsis sp.(Sacc) Bubák (226)	-	1,08	-
Phomopsis sp.(Sacc) Bubák (43)	2,22	-	-
Pleosporales Luttr. ex M.E. Barr (126)	1	1,46	+++
Pleosporales Luttr. ex M.E. Barr (141)	2,18	-	++
Pleosporales Luttr. ex M.E. Barr (149)	1,84	1	++
Preussia africana Arenal, Platas & Peláez (105)	1,5	-	-
Preussia africana Arenal, Platas & Peláez (136)	0,63	1	+++
Preussia africana Arenal, Platas & Peláez (33)	1	-	-
Preussia australis (Speg.) Arx (2)	1	-	-
Preussia minima (Auersw.) Arx (154)	0,72	-	++
Preussia sp. Fuckel (100)	1	-	+++
Preussia sp. Fuckel (102)	1	-	-
Preussia sp. Fuckel (103)	0,75	-	-
Preussia sp. Fuckel (124)	0,61	-	++
Sarcosomataceous Kobayasi (155)	0,92	0,83	-
Sclerostagonospora opuntiae (Ellis & Everh.) Huhndorf (115)	1	1,42	++
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not. (135)	1	-	-
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not. (37)	0,7	1	++
Sporormiella sp. Ellis & Everh. (108)	-	-	+++
Trichoderma saturnisporum Hammill (210)	0,59	1,31	-
Xylaria acuta Peck (151)	1	1,11	-
Xylaria acuta Peck (228)	2,03	1,85	+++
Xylaria digitata (L.) Grez. (225)	1,63	1,73	+
Xylaria enteroleuca (Speg.) P.M.D. Martin (134)	1,35	-	++
Xylaria hypoxylon (L.) Grev. (28)	-	1	+++
Xylaria sp. Hill ex Schrank (122)	1,33	1,82	++
Xylaria sp. Hill ex Schrank (215)	2,52	1	-
Xylaria sp. Hill ex Schrank (63)	1,07	-	++++
Xylariales (Amphisphaeriaceae) G. Winter (101´)	1	3,13	+
Xylariales (Amphisphaeriaceae) G. Winter (110)	1,88	1,96	-
Xylariales (Amphisphaeriaceae) G. Winter (143)	2,22	3,29	++
Xylariales (Xylariaceae) Tul. & C. Tul. (120)	-	1,61	++++
Xylariales (Xylariaceae) Tul. & C. Tul. (133)	1	1,05	+++
Sterile mycelium (153)	1,06	-	+++
Sterile mycelium (160)	-	-	++
Sterile mycelium (148)	1,22	1	++++

Sterile mycelium (233)	1	1,11	-
Sterile mycelium (156)	1,33	1	-
Sterile mycelium (235)	1,22	-	-
Sterile mycelium (40)	-	-	+
Nº isolates with EA >1	**48**	**46**	**21**
Nº isolates with EA >0	**86**	**62**	**53**
% isolates with EA >1	**47%**	**45%**	**20%**
% isolates with EA >0	**83%**	**60%**	**51%**

EA: enzymatic activity (clearance index). Discoloration by enzyme activity was evaluated from negative (-), light (+) positive reaction and positive reaction increasing from (++) to (++++).

Table 2. Chitinolytic and proteolytic activity of *M. anisopliae*

Entomopathogenic endophytic fungi	**Incubation time (days)**	**Proteases (AE)**	**Chitinases (AE)**
Metarhizium anisopliae (Metschn.) Sorokīn (238)	2	**1,86**	-
	7	1,28	1,11
	14	-	**1,16**

Table 3. Microbial growth inhibition produced by fungal metabolites (inhibition halo in mm)

Fungal endophytes	**Patogenic bacteria**				**Patogenic fungi**			
	E. c.	***S. a.***	***X. c.***	***Er. c.***	***C. a.***	***C. g.***	***A. f.***	***F. o.***
Alternaria alternata Keissl (47)	-	4	-	-	-	-	-	-
Alternaria alternata Keissl (101)	-	2,5	-	-	-	-	-	-
Colletotrichum gloeosporioides (Penz) Penz & Sacc (205)	-	5	0,5	-	-	-	-	-
Coniochaeta velutina (Fuckel) Cooke (221)	0,5	1	-	-	-	-	-	-
Daldinia sp. Ces. & De Not. (241)	0,5	0,5	-	-	-	-	-	1
Lentinus tigrinus (Bull.) Fr. (207)	-	-	1	-	-	-	-	-
Lophiostoma sp. Ces & De Not (246)	1	10	1	1	2,5	2	2	1
Lophiostoma sp. Ces & De Not (57)	2	2	-	-	-	-	-	-
Pestalotiopsis guepinii (Desm) Steyaert (104)	-	1,5	-	-	-	-	-	-
Phomopsis sp. (Sacc) Bubák (203)	-	1	-	-	-	-	-	-
Phomopsis sp. (Sacc) Bubák (21)	-	0,8	-	-	-	-	-	-
Preussia africana Arenal, Platas & Peláez (33)	1	1	2	-	-	-	-	-
Preussia africana Arenal, Platas & Peláez (33')	1	1	3,5	-	-	-	-	-
Preussia australis (Speg.) Arx (17)	1	1	-	-	-	-	-	-
Sclerostagonospora opuntiae (Ellis & Everh.) Huhndorf (115)	-	1	-	-	-	-	-	-
Sclerostagonospora opuntiae (Ellis & Everh.) Huhndorf (115')	-	-	-	-	1	-	-	-
Sclerostagonospora opuntiae (Ellis & Everh.) Huhndorf (145)	-	3	-	-	-	-	-	-
Xylaria digitata (L.) Grev. (225)	-	-	-	0,5	-	-	-	-
Sterile mycelium (235)	-	-	0,5	-	1	-	-	-
Negative control (acetone)	-	-	-	-	-	-	-	-
Positive control- ampicilin 0,2 g/ml	15	37	15	29	-	-	-	-
Positive control - ketoconasol (2%)	-	-	-	-	14	10	8	3

Table 4. Phytotoxicity of extracts from endophytes

Fungal isolates	**Origin**		**Phytotoxicity**
	Plant	**Organs**	**Lesion**
Alternaria alternata (Fr.) Keissl. (101)	Mc	blade	-
Alternaria alternata (Fr.) Keissl. (47)	Ma	xylem	-
Colletotrichum acutatum J.H. Simmonds (50)	Ma	xylem	+
Colletotrichum gloeosporioides (Penz) Penz & Sacc. (1')	Ma	petiole	-
Colletotrichum gloesporioides (Penz) Penz & Sacc. (205)	Eu	blade	-
Diaporthe sp. Nitschke (224)	Eu	petiole	+
Glomerella cingulata (Stoneman) Spauld & Schrenk (223)	Eu	blade	-
Guignardia mangiferae A. J. Roy (116)	Mc	petiole	+
Neofusicoccum australe (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips (132)	Mc	petiole	+
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips (12)	Ma	petiole	-
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips (205')	Eu	blade	-
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips (212)	Eu	blade	+
Nigrospora sphaerica (Sacc) Mason (113)	Mc	petiole	++
Phaeoacremonium sp. W. Gams, Crous & M.J. Wingf. (20)	Ma	petiole	+
Phaeoacremonium sp. W. Gams, Crous & M.J. Wingf. (243)	Eu	xylem	-
Phomopsis sp.(Sacc) Bubák (202)	Eu	blade	-
Phomopsis sp.(Sacc) Bubák (203)	Eu	blade	-
Phomopsis sp.(Sacc) Bubák (204)	Eu	blade	-
Phomopsis sp.(Sacc) Bubák (206)	Eu	blade	+
Phomopsis sp.(Sacc) Bubák (21)	Ma	blade	-
Phomopsis sp.(Sacc) Bubák (213)	Eu	petiole	-
Phomopsis sp.(Sacc) Bubák (214)	Eu	petiole	-
Phomopsis sp.(Sacc) Bubák (217)	Eu	petiole	-
Phomopsis sp.(Sacc) Bubák (219)	Eu	petiole	-
Phomopsis sp.(Sacc) Bubák (226)	Eu	corteza	-
Preussia sp. Fuckel (100)	Mc	blade	-
Xylaria digitata (L.) Grez. (225)	Eu	blade	-
Xylaria sp. Hill ex Schrank (120)	Mc	blade	++
Xylariales (Amphisphaeriaceae) G. Winter (110)	Mc	petiole	-

(-) No injury
(+) Very slight injury around the punctures
(++) Significant injury throughout the area applied
Mc., *Myrcianthes cisplatensis*; Ma., *Myrrhinium atropurpureum*; Eu., *Eugenia uruguayensis*