The foliar fungal endophytes of *Citrus limon* in Argentina

Estela L. Durán, L. Daniel Ploper, Juan C. Ramallo, Rosely A. Piccolo Grandi, Ágata C. Hupper Giancoli, and João L. Azevedo

Abstract: Fungal endophytes associated with *Citrus limon* (L.) Burm., were investigated. Culture of surface-sterilized leaves from two orchards of lemon trees in Tucumán, located in northwestern Argentina, revealed the presence of endophytic fungi in all leaves examined. The colonization frequencies were 69.7% and 72.3% at the two sampled sites, and a total of nine taxa were isolated. Eight of the taxa had relative frequencies of >2% in at least one sample. The number of isolates was significantly lower in the spring. The assemblage composition at each site and sampling time was similar. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., responsible for anthracnose, was consistently dominant. Another pathogenic fungus of *Citrus* spp., *Guignardia citricarpa* Kiely was also recovered as an endophyte. Scanning electron micrographs of cut uncultured and cultured leaf surfaces, seen in transverse section, showed intercellular fungal hyphae in parenchyma cells No penetration of host cells by either fungus was observed. This is the first report on fungal endophytes in leaves of lemon plants in Argentina.

Key words: endophytic fungi, lemon, colonization, scanning electron microscopy.

Résumé : Les auteurs ont étudié les endophytes fongiques associés au *Citrus limon* (L.) Burm. La mise en culture de feuilles superficiellement stérélisées, provenant de deux citronniers poussant à Tucumán dans le nord-ouest de l'Argentine, révèle la présence de champignons endophytes dans toutes les feuilles examinées. Les fréquences de colonisation sont 69,7 % et 72,3 % pour les deux sites échantillonnés, avec un total de neuf taxons. Huit des neuf taxons montrent des fréquence relatives de plus de 2 %, dans au moins un des échantillons. Le *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., responsable de l'anthracnose, est constamment dominant. On retrouve également comme endophyte, un autre champignon pathogène des *Citrus* spp., le *Guignardia citricarpa* Kiely. Les micrographies en microcopie électronique par balayage, obtenues de surfaces foliaires en coupe transversale, en culture ou non, montrent des hyphes fongiques intercellulaires dans les cellules de parenchyme. On observe aucune pénétration de cellules hôtes, par l'un ou l'autre des champignons. Il s'agit du premier rapport sur la présence d'endophytes fongiques dans les feuilles de citronniers en Argentine.

Mots clés : champignons endophytes, citron, colonisation, microscopie électronique par balayage.

[Traduit par la Rédaction]

Introduction

Argentina is the major world exporter of lemons and 95% of the lemon crop is produced in the province of Tucumán. The citrus industry and associated activities generate US$300 million each year in this region and have created numerous jobs. Citrus black spot, caused by *Guignardia citricarpa* Kiely, is an important fruit disease that is widespread in most citrus-growing areas of the world. Affected fruits are unacceptable for the fresh fruit market (Kotzé 1981, 1988). In Argentina *G. citricarpa* was first reported by Marchionatto (1928), but only in recent years has this fungus received major attention because these pathogenic strains are now subject to quarantine legislation in the United States and the European Union. Lemon exports have been increasing because of the imposed strict controls once it is demonstrated that the area is free of serious pests and diseases of quarantine significance, including citrus black spot. There is an increased pressure on Argentinian lemon growers to produce more certified healthy fruits.

Fungal endophytes can colonize internal plant tissues without causing apparent harm to their host. This definition is applied not only to mutualistic and neutral endophytes, but also to those that have an epiphytic phase and to latent pathogens that may live symptomless in their hosts for some part of their life cycle (Petrini 1991). Fungal endophytes are being recognized for their potential role as biological control agents and as metabolite sources of medical and agricultural importance (Azevedo et al. 2000). Endophytes are widespread in many plants. Most investigations into their occurrence have involved trees and shrubs of north-temperate

Received 13 February 2004. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 31 March 2005.

E.L. Durán,[1] L.D. Ploper, and J.C. Ramallo. Cátedra de Fitopatología, Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Avenida Roca 1900, (4000) Tucumán, Argentina.
R.A. Piccolo Grandi. Instituto de Botânica, Seção de Micologia e Liquenologia, Caixa Postal 4005, 01061-970, São Paulo, SP, Brasil.
Á.C. Hupper Giancoli and J.L. Azevedo. Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Av. Pádua Dias 11, Caixa Postal 83, 113400-970 Piracicaba, SP, Brasil.

[1]Corresponding author (e-mail: eslidu@fbqf.unt.edu.ar).

 doi: 10.1139/B05-009

regions (eg., Carroll and Carroll 1978; Carroll 1988; Petrini 1986; Petrini and Fisher 1990; Collado et al. 1999), and only a few investigations on fungal endophytes in citrus plants have been conducted. *Guignardia citricarpa* was the first fungus to be reported as an endophyte in healthy *Citrus* spp. (Shüepp 1961), followed by *Physoderma citri* (Childs et al. 1965). More recently several authors (Wright et al. 1998; Araujo et al. 2001; Glienke-Blanco et al. 2002) isolated endophytes from different citrus plants, the genera *Colletotrichum*, *Guignardia*, and *Cladosporium* being the most frequently found.

Since endophytic fungi in healthy citrus tissues may represent a reservoir of quiescent pathogens, potential pathogens, pathogen antagonists, insect repellents, harmless saprophytes, and host defense elicitors (Wright et al. 1998), research is needed to evaluate precise benefits associated with these endophytes on lemon crops.

Although many cultural studies have demonstrated the presence of endophytic fungi in host plants, less is known about the host–fungus relationship at the ultrastuctural level (Bernstein and Carroll 1977; Stone 1988; Johnson and Whitney 1989; Viret and Petrini 1994; Deckert et al. 2001).

The aims of this work were (*i*) to isolate and identify the endophytic fungi in leaves of *Citrus limon*, (*ii*) to study the influence of geographical and seasonal factors on fungal communities, and (*iii*) to investigate location, size, and shared characteristics of endophytic fungi within leaf tissues using scanning electron microscopy.

Materials and methods

Collection of samples

Leaves were sampled from two lemon (*Citrus limon* (L.) Burm.) orchards, located at El Manantial and Yacuchina, in Tucumán, a province in northwestern Argentina. The distance between El Manantial and Yacuchina is 55 km. Both orchards are in the citrus-growing area of the province, characterized by its subtropical climate, with an average isotherm value of 16–19 °C and frost-free periods from September through April. The average temperatures for January (summer) and July (winter) are 25 and 12 °C, respectively. Average annual rainfall is 800–1200 mm; rains are confined to the summer months; the winter months are very dry.

The diversity of endophytic fungi was determined in leaves of 'Volkameriano' (*Citrus volkameriana* Pascuale) and Citrumelo 'Swingle' (*Citrus paradise* × *Poncirus trifoliata*) lemons, collected from orchards at El Manantial and Yacuchina, respectively. Sampled trees were more than 5 years old at the beginning of the study. The third or fourth leaf, from branches located in the external part of the plant, was chosen at a height of approximately 1.50 m. A total of 128 healthy looking leaves were collected from eight plants from each orchard in the first week of November 1997, and February, May, and August 1998. A preliminary sampling was collected in September 1997 to obtain data on the diversity of endophytic mycota and to test the methods of analysis. Samples were brought to the laboratory in sterile polyethylene bags and stored in a refrigerator at 6 °C for no longer than 24 h prior to use.

Isolation of endophytes

The leaves were washed thoroughly in running water and sterilized using the following immersion sequence: 1 min in 96% ethanol, 4 min in a solution of NaOCl (4% *v*/*v* available chlorine), and 30 s in 96% ethanol. The optimal length of each step was determined in preliminary tests by modifying the protocols described by Petrini (1986). The leaves were then cut with a sterile scalpel into five segments of 0.5 cm^2 and transferred in serial order to 90-mm Petri plates containing solid complete medium (CM) as described by Pontecorvo et al. (1953). Penicillin G (20 $U \cdot mL^{-1}$) and streptomycin sulphate (40 $mg \cdot mL^{-1}$) were added to suppress bacterial growth. Plates were incubated at 28 °C for 21 d under a 12 h fluorescent light : 12 h dark cycle and examined periodically for 5–21 d. Outgrowing mycelia were isolated, purified, and transferred onto slants containing CM without antibiotics.

Identification and nomenclature

Identification of fungal taxa was based on both cultural characteristics and morphology of fruiting bodies and spores. Most fungi sporulated readily on the leaf tissue but only slowly on artificial medium. Sterile isolates were described as "morphospecies" and differentiated by cultural characteristics. Cultures of the representative isolates are being maintained in our laboratory.

Statistical analysis

Colonization frequency (CF) of endophyte species was determined using the method of Fisher and Petrini (1987):

$$CF = (N_i/N_t) \times 100$$

where N_i is the number of segments from which the fungus was isolated and N_t is the total number of segments cultured.

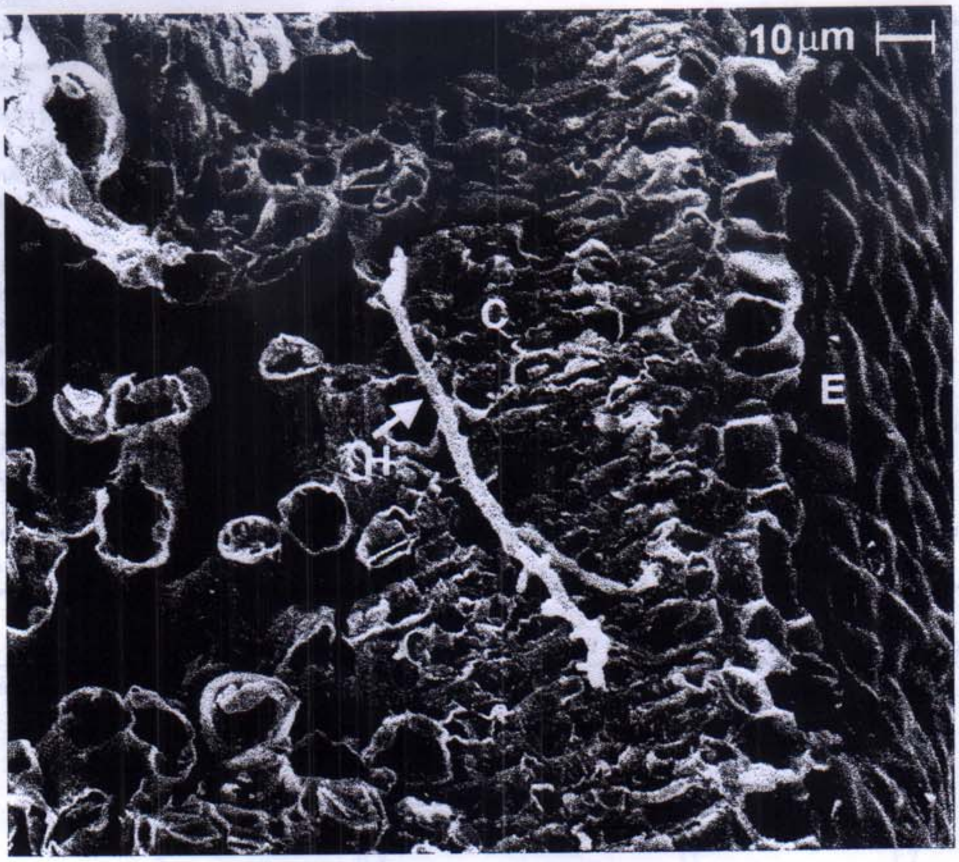
To compare differences in the numbers of fungal endophytes isolated at the two sites and among trees for each site, the number of fungal endophytes was square-root transformed and compared using analysis of variance. Variations in the number of fungal endophytes found in the four seasons were analyzed using Tukey's test. An α value of 0.05 was used to assess significance. The statistical package SAS version 6.0 (SAS Institute Inc, Cary, N.C) was used for analyses of variance and Tukey's tests.

Scanning electron microscopy

Sixteen leaves for scanning electron microscopy (SEM) were surface sterilized and cross-sectioned with a razor blade into segments of approximately 0.2 cm^2. Five segments from each leaf were placed on CM and incubated at 28 °C under a 12 h fluorescent light : 12 h dark cycle; another adjacent segment was fixed for SEM less than 24 h after removal from the tree.

Cultured and uncultured fresh leaf segments were placed in Karnovsky's fixative for 2 h and postfixed for 1 h in a 1% osmium tetroxide solution. The segments were rinsed in distilled water, dipped in 30% glycerol solution for 15 min, and then immersed in liquid nitrogen for 30 min, followed by fracture with a scalpel. The samples were transferred to a 0.1% osmium tetroxide solution for 3 d, were dehydrated in an acetone series (30%, 50%, 70%, 90%, 100%), and processed in a Balzers CPD 050 critical-point drier (Balzers

Fig. 1. Scanning electron micrograph of the cut surface of an uncultured segment of *Citrus limon* leaf showing the internal fungi. A fungal hypha (H) is growing adhered to the surface of palisade parenchyma cells (C). E, epidermis.



Union Ltd., Liechtenstein). Upon removal from the drier, specimens were mounted, cut edge up on stubs with conductive silver paint, and coated with gold in a Balzers MED 010 sputter coater. A LEO 435 VP scanning electron microscope (Thornwood, New York) linked to a computer operating LEOUIF version 3.01 software was used to observe the specimens. The images obtained were stored in a computer.

Results and discussion

Microscopy

Cultured and uncultured fresh leaf segments were examined by SEM for a visual assessment of size, shape, and location of fungal hyphae within a leaf. The direct electron microscopic observation of ultrastructural aspects of the interaction between a particular endophyte and its host often presented problems. Internal fungi were seen in four out of sixteen uncultured fresh segments examined. A scanning electron micrograph of a cut edge of uncultured segment of leaf (Fig. 1) shows fungal hypha that is lying across palisade parenchyma cells. Mycelial penetration of living cells was not observed; infections appeared to be entirely intercellular. The scanning electron micrographs of cut cultured leaves show fungal hyphae growing only between cells of the leaf parenchyma; the fungal hyphae were never seen to lie within the epidermal tissue.

Colonization frequencies

The leaves of all collected material harboured endophytic fungi. A total of 909 isolates were obtained between November 1997 and August 1998 from 1280 leaf segments collected from orchards at El Manantial and Yacuchina. The number of isolates and relative colonization frequencies are given in Table 1. Overall, the total number of isolates from the orchard at El Manantial did not differ significantly ($p < 0.05$) from the total of the orchard at Yacuchina. Leaf segments from both sites had the lowest colonization frequencies in the spring (November 1997), with no differences in the other three seasons. The analysis of variance did not reveal significant differences ($p < 0.05$) among the number of fungal isolates from each tree sampled at each site (data not shown).

To our knowledge, this is the first report of endophytes isolated from *Citrus limon* in Argentina. The presence of endophytes in citrus plants is consistent with the reports by Wright et al. (1998), Araujo et al. (2001), and Glienke-Blanco et al. (2002), which showed that endophytic fungi can be isolated from leaves of *Citrus* spp. The fungal colonization frequencies obtained from lemon leaves (72.3% and 69.7% from orchards at El Manantial and Yacuchina, respectively) are lower than those reported for the four varieties of tangerine plants in Brazil (81%) (Glienke-Blanco et al. 2002). The presence of young leaves in the spring sample may be the cause of this disparity. Several studies confirm that infections increase with leaf age (Bernstein and Carroll 1977; Rodrigues 1992). This increase could be explained by

Table 1. Number and percentage of colonized segments of *Citrus limon* leaves according to site and sampling date.

Sample	No. of colonized segments*	% of colonized segments†
El Manantial		
November 1997	70	43.8a
February 1998	134	83.8b
May 1998	125	78.1b
July 1998	134	83.8b
Total	463	72.3b
Yacuchina		
November 1997	92	57.5a
February 1998	123	76.9b
May 1998	110	68.7b
July 1998	121	75.6b
Total	446	69.7b

*160 leaf segments were used for each sample, giving a total of 1280 sampled segments.
†Percentages followed by the same letter are not significantly different according to Tukey's test ($p > 0.05$).

Table 2. Number of isolates and relative colonization percentage of each taxa recovered from healthy *Citrus limon* (lemon) leaves in El Manantial and Yacuchina.

Taxon	El Manantial No. of isolates	El Manantial % of segments colonized	Yacuchina No. of isolates	Yacuchina % of segments colonized
Colletotrichum gloeosporioides	298	46.6	222	34.7
Guignardia citricarpa	4	0.6	19	3.0
Alternaria alternata	25	3.9	14	2.2
Nigrospora sphaerica	38	5.6	46	7.2
Nodulisporium sp.	20	3.1	0	0.0
Sporormiella minima	0	0.0	14	2.2
Xylariaceae	6	0.9	11	1.7
Sterile white mycelium	55	8.6	66	10.3
Sterile pigmented mycelium	17	2.7	54	8.4

Note: 640 leaf segments were analysed from each locality.

the high genetic diversity among our isolates of lemon leaves, reveled by random amplified polymorphic DNA (RAPD), suggesting a constant 'new infection' of the same leaf (Durán 2001).

Composition of endophytic assemblage

Although only nine endophytic taxa were isolated from leaves of *Citrus limon*, most of them showed a colonization frequency of more than 2% in at least one of the sites. The number of isolates and the relative colonization frequency of each fungal taxon are listed in Table 2.

Araujo et al. (2001) reported high frequencies of the genera *Colletotrichum*, *Guignardia*, and *Cladosporium* in eight citrus rootstocks commonly used in Brazil. Glienke-Blanco et al. (2002) also reported that relatively few taxa account for a large number of isolates from tangerine plants; the most common fungi recovered were *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (60%) and *Guignardia* (27%) from which 11% belonged to the species *G. citricarpa*.

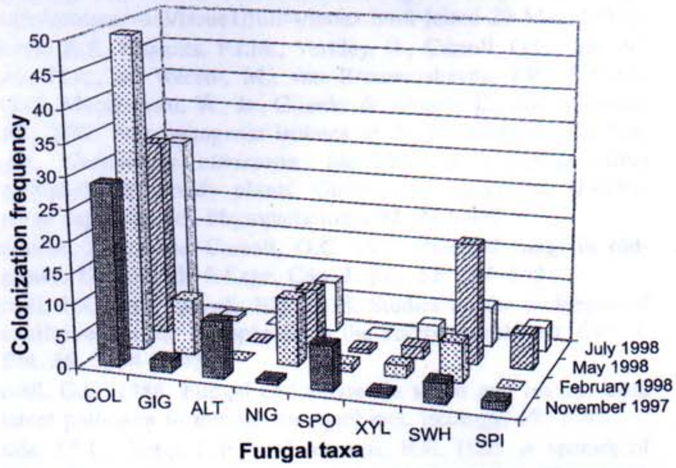
In our study *Colletotrichum gloeosporioides* also represented the majority of isolates (34.7%–46.6%), but the frequency of *G. citricarpa* was lower (0.6%–3%). Wright et al. (1998) suggested that agricultural practices in monoculture orchards lead to a loss in fungal diversity and the prevalence of pathogenic fungi such as *Colletotrichum gloeosporioides*.

Of all taxa from *Citrus limon* leaves, *Colletotrichum gloeosporioides* and sterile white mycelia were repeatedly obtained from both sites in all four seasons (Fig. 2). Several endophytes, such as *G. citricarpa*, *Alternaria alternata*, *Nigrospora sphaerica*, sterile pigmented mycelium, and species of Xylariaceae, were associated with *Citrus limon* irrespective of geographical origin, but not in all seasons. Together, these fungi composed 14%–24% of the endophyte assemblages. *Nodulisporium* sp. and *Sporormiella minima* were recovered from only one orchard, El Manantial or Yacuchina, respectively, and could be considered as site-dependent occasionals.

In the present study, endophyte composition in lemon leaves did not show different seasonal patterns, confirming the observations by Petrini (1991) for other hosts. The most common endophytes were recovered throughout the year, but higher colonization frequencies were obtained in the summer and winter samples.

The fungal taxa recovered as *Citrus limon* endophytes have also been recovered from a variety of tropical hosts including *Musa acuminata* (Pereira et al. 1999), *Euterpe oleracea* (Rodrigues 1992), and *Rhizophora* spp. (Suryanarayanan et al. 1998).

Fig. 2. Comparison of the relative frequencies of colonization of *Citrus limon* by eight fungal endophyte taxa from Yacuchina at each sampling date. COL, *Colletotrichum gloeosporioides*; GIG, *Guignardia citricarpa*; ALT, *Alternaria alternata*; NIG, *Nigrospora sphaerica*; SPO, *Sporormiella minima*; XYL, Xylariaceae; SWH, sterile white mycelium; SPI, sterile pigmented mycelium.



Colletotrichum gloeosporioides, responsible for anthracnose on citrus, was the most frequently isolated endophyte from healthy (symptomless) plants in the two orchards. Latent infections by *Colletotrichum gloeosporioides* do occur in *Citrus* spp. A true latent infection must involve a parasitic relationship that eventually induces symptoms. The infection hyphae of *Colletotrichum gloeosporioides* can be produced from appressoria. Sometimes these appressoria remain viable on the surface of the host, but infections have not yet taken place (Verhoeff 1974). Accordingly, it is possible that isolates of *Colletotrichum gloeosporioides* were obtained from dormant appressoria and were not endophytes. This fungus could be considered a latent pathogen since studies carried out to prove pathogenicity of isolates did not exclude the possibility that the endophytic isolates of *Colletotrichum gloeosporioides* are pathogenic (Durán 2001). Since effective development of anthracnose depends so much on lowered vitality of tree tissues (Agrios 1991), the occurrence of anthracnose in lemon orchards is probably a consequence of a previous endophytic infection in the field. On the other hand, Garcia (1996) reported that anthracnose is of minor importance in *Citrus limon* from Tuçumán, although the disease is commonly found in the majority of lemon plantings. Schulz et al. (1999) developed the hypothesis that both the host–pathogen and the host–endophyte interactions involve constant mutual antagonisms based, in part, on the secondary metabolites that partners produce. Whereas the host–pathogen interaction is unbalanced and results in disease, that of the endophyte and its host is a balanced antagonism. The occurrence of fungal pathogens in healthy plant tissues has also been reported from subtropical fruits (Verhoeff 1974), *Alnus rubra* (Sieber et al. 1991), *Euterpe oleracea* (Rodrigues 1992), and *Trachycarpus fortunei* (Taylor et al. 1999).

The fungus *G. citricarpa*, which causes citrus black spot, was isolated as an endophyte from asymptomatic lemon plants. Endophytic nonpathogenic isolates, morphologically indistinguishable from pathogenic strains, were differentiated by RAPD and polymerase chain reactions (Durán 2001). Based on genetic variability data, we confirmed that the isolates from healthy plants in the present study were nonpathogenic. Further support for this assumption comes from (*i*) reports by McOnie (1964) that *Citrus* spp. and many other host species might harbor a latent nonpathogenic *Guignardia* sp. that is morphologically similar to pathogenic strains of *G. citricarpa*; and (*ii*) RAPD data (Glienke-Blanco 1999). Recently Baayen et al. (2002), using ITS sequence analysis, revealed two phylogenetically distinct groups in *G. citricarpa*. Furthermore, they suggested that nonpathogenic isolates are not strains or physiologically specialized forms of *G. citricarpa*, but rather a separate species, *Guignardia mangiferae*.

Of the 17 Xylariaceae isolates recovered, nine were sterile and eight were present only as the anamorph. Species identification was difficult because teleomorphs were never formed, and most keys are based only on teleomorphic features. Xylariaceae endophytes from *Citrus limon* were identified using a key constructed by Petrini et al. (1995) based on cultural and anamorphic features. Four "morphospecies" were isolated from lemon leaves, suggesting the existence of Xylariaceae species diversity within plants or leaves. Xylariaceae endophytes were isolated from lemon leaves, sometimes at low frequencies, from both study sites. Their regular occurrence in lemon trees from Yacuchina suggests that they could be of some significance to the colonized host and even influence its physiology. Xylariaceae are common endophytes, especially in tropical plants, and have been previously reported from *Alnus rubra* (Sieber et al. 1991) and *Euterpe oleracea* (Rodrigues 1992).

The role of endophytic fungi in *Citrus limon* is still unknown. The biology of these apparently endophytic isolates deserves to be explored. The presence of endophytic fungi in asymptomatic lemon plants and the long-lived association indicate that further investigations are needed to evaluate the advantages of such associations and their potential for development as biocontrol agents or as vectors of biological control agents.

Acknowledgements

The authors gratefully thank Dra. Aline Pizzirani-Kleiner, who provided laboratory facilities at Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil. We also thank Dr. Elliot W. Kitagima (NAP/MEA, ESALQ/USP, Piracicaba, São Paulo, Brazil) for the use of the scanning electron microscope. Thanks are also due to Dr. Isaías Geraldi and Ing. Agr. Alberto Manlla for statistical analysis. This research was supported by Consejo de Investigaciones de la Universidad Nacional de Tucumán Program No. 26/A215 and Consejo Nacional de Investigaciones Científicas y Técnicas No. 4960/96.

References

Agrios, G.N. 1991. Fitopatología. Editorial Limusa Sociedad Anónima, Mexico D.F.

Araujo, W.L., Maccheroni, W., Jr., Aguilar-Vildoso, C.I., Barroso, P.A V., Saridakis, N.O., and Azevedo, J.L. 2001. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can. J. Microbiol. **47**: 229–236.

Azevedo, J.L., Maccheroni, W., Jr., Pereira, J.O., and Araujo, W.L. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic J. Biotechnol. [online], **3**(1): 40–65. Available from http://www.ejbiotechnology.info/content/vol3/issue1/full/4/index.html [cited 20 May 2003].

Baayen, R.P., Bonants, P.J.M., Verkley, G., Carroll, G.C., van der Aa, H.A., de Weerdt, M., van Brouwershaven, I.R., Schutte, G.C., Maccheroni, W., Jr., Glienke de Blanco, C., and Azevedo, J.L. 2002. Nonpathogenic isolates of the citrus black spot fungus, *Guignardia citricarpa*, identified as a cosmopolitan endophyte of woody plants, *Guignardia mangiferae* (*Phyllosticta capitalensis*). Phytopathology, **92**: 464–477.

Bernstein, M.E., and Carroll, G.C. 1977. Internal fungi in old-growth Douglas fir foliage. Can. J. Bot. **55**: 644–653.

Carroll, G.C., and Carroll, F.E. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can. J. Bot. **56**: 3034–3043.

Carroll, G.C. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology, **69**: 2–9.

Childs, J.F.L., Kopp, L.E., and Johnson, R.E. 1965. A species of *Physoderma* present in citrus and related species. Phytopathology, **55**: 681–687.

Collado, J., Platas, G., González, I., and Peláez, F. 1999. Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. New Phytol. **144**: 525–532.

Deckert, R.J., Melville, L.H., and Peterson, R.L. 2001. Structural features of a *Lophodermium* endophyte during the cryptic life-cycle phase in the foliage of *Pinus strobus*. Mycol. Res. **105**: 991–997.

Durán, E.L. 2001. Aislamiento, identificación y diversidad genética de hongos endofíticos de limón. Ph.D. thesis, Department of Microbiology, Universidad Nacional de Tucumán, Tucumán, Argentina.

Fisher, P.J., and Petrini, O. 1987. Location of fungal endophytes in tissues of *Suaeda fruticosa*: a preliminary study. Trans. Br. Mycol. Soc. **89**: 246–249.

Garcia, M.A. 1996. Plantación y mantenimiento de la quinta. *In* Manual de producción de limón. *Edited by* M.O. Haro. Instituto Nacional de Tecnología Agropecuaria EEA Famaillá, Tucumán, Argentina. pp. 113–190.

Glienke-Blanco, C. 1999. *Guignardia citricarpa* Kiely: Análise genética, cariotípica e interação com o hospedeiro. Ph.D. thesis, Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil.

Glienke-Blanco, C., Aguilar-Vildoso, C.I., Carneiro-Vieira, M.L., Barroso, P.A.V., and Azevedo, J.L. 2002. Genetic variability in the endophytic fungus *Guignardia citricarpa* isolated from citrus plants. Genet. Mol. Biol. **25**: 251–255.

Johnson, J.A., and Whitney, N.J. 1989. A study of fungal endophytes of needles of balsam fir (*Abies balsamea*) and red spruce (*Picea rubens*) in New Brunswick, Canada, using culture and electron microscope techniques. Can. J. Bot. **67**: 3513–3516.

Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. Plant Dis. **65**: 945–950.

Kotzé, J.M. 1988. Black spot. *In* Compendium of citrus diseases. *Edited by*. J.O. Whiteside, S.M. Garnsey, and L.W. Timmer. The American Phytopathological Society, St. Paul, Minn. pp. 10–12.

Marchionatto, J.B. 1928. Fitoparásitos de la Argentina nuevos o poco conocidos. II. Physis, **32**: 145.

McOnie, K.C. 1964. The latent occurrence in citrus and other hosts of a *Guignardia* easily confused with *G. citricarpa*, the citrus black spot pathogen. Phytopathology, **54**: 40–43.

Pereira, J.O., Carneiro-Vieira, M.L., and Azevedo, J.L. 1999. Endophytic fungi from *Musa acuminata* and their reintroduction into axenic plants. World J. Microbiol. Biotechnol. **15**: 37–40.

Petrini, O. 1986. Taxonomy of endophytic fungi of aerial plant tissues. *In* Microbiology of the phyllosphere. *Edited by* N.J. Fokkema and J. van den Heuvel. Cambridge University Press, Cambridge, UK. pp. 175–187.

Petrini, O. 1991. Fungal endophytes of tree leaves. *In* Microbial ecology of leaves. *Edited by* J.H. Andrews and S.S. Hirano. Springer-Verlag, New York. pp. 179–197.

Petrini, O., and Fisher, P.J. 1990. Ocurrence of fungal endophytes in twigs of *Salix fragilis* and *Quecus robur.* Mycol. Res. **94**: 1077–1080.

Petrini, O., Petrini, L. E., and Rodrigues, K. F. 1995. Xylariaceous endophytes: an exercise in biodiversity. Fitopatol. Bras. **20**: 531–539.

Pontecorvo, G., Roper, J.A., Hemmons, L.M., MacDonald, K.D., and Bufton, A.W.J. 1953. The genetics of *Aspergillus nidulans*. Adv. Genet. **5**: 141–238.

Rodrigues, K.F. 1992. Endophytic fungi in the tropical palm *Euterpe oleracea* Mart. Ph.D. thesis, Department of Botany, The City University of New York, New York.

Schulz, B., Römmert, A.-K., Dammann, U., Aust, H.-J., and Strack, D. 1999. The endophyte-host interaction: a balanced antagonism? Mycol. Res. **103**: 1275–1283.

Sieber, T.N., Sieber-Canavesi, F., and Dorworth, C.E. 1991. Endophytic fungi of red alder (*Alnus rubra*) leaves and twigs in British Columbia. Can. J. Bot. **69**: 407–411.

Shüepp, H. 1961. Untersuchungen über *Guignardia citricarpa* Kiely, den erreger der scharzfleckenkrankheit auf citrus. Phytopathol. Z. **40**: 258–271.

Stone, J.K. 1988. Fine structure of latent infections by *Rhabdocline parkeri* on Douglas-fir with observations on unifected epidermal cells. Can. J. Bot. **66**: 45–54.

Suryanarayanan, T.S., Kumaresan, V., and Johnson, J.A. 1998. Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Can. J. Microbiol. **44**: 1003–1006.

Taylor, J.E., Hyde, K.D., and Jones, E.B.G. 1999. Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei*, within and outside its natural geographic range. New Phytol. **142**: 335–346.

Verhoeff, K. 1974. Latent infections by fungi. Annu. Rev. Phytopathol. **12**: 99–110

Viret, O., and Petrini, O. 1994. Colonization of beech leaves (*Fagus sylvatica*) by the endophyte *Discula umbrinella* (teleomorph: *Apiognomonia errabunda*). Mycol. Res. **98**: 423–432.

Wright, J.G., Johnson, G.I., and Hyde, K.D. 1998. Studies on the endophytic mycota of *Citrus* spp. *In* Disease Resistance in Fruit. Proccedings of the International Workshop held at Chiang Mia, Thailand, 18–21 May 1997. *Edited by* G.I. Johnson, E. Highley, and J. Joyce. The Australian Centre for International Agriculture Research (ACIAR), Canberra, Australia. No. 80. pp. 167–173.