Fungal survey for biocontrol agents of *Ipomoea carnea* from Brazil

D.J. Soares and R.W. Barreto

Summary

Ipomoea carnea Jacq., also known as morning glory, is native of tropical America, and its purported centre of origin is the Paraguay Basin. This plant is feared by ranchers because of its well-documented toxicity to cattle. Because of its showy flowers, it became a popular ornamental in Brazil and was introduced into others countries, becoming an aggressive wetland ecosystem invader. Little is known about its mycobiota in Brazil which may include fungal pathogens that could be used in classical biocontrol programmes. *Coleosporium ipomoeae* (Schwein.) Burril and *Puccinia puta* H.S. Jacks. and Holw. ex F. Kern, Thurst. and Whetzel are the only fungi recorded in the literature attacking this plant in Brazil. An intensive search for specialized, coevolved fungal pathogens of *I. carnea* was initiated in 2003 in Brazil. Twenty-one fungal species were collected. Among these were the two previously known rusts, *C. ipomoeae* and *P. puta*, and *Aecidium* sp., *Albugo* sp., an unidentified *ascomycete*, *Mycosphaerella* sp., five coelomycetes (*Colletotrichum* sp., *Phoma* sp. *Phomopsis* sp., and two *Phyllosticta* spp.) and ten hyphomycetes (*Alternaria* sp., *Cercospora* sp., *Cladosporium* sp., *Curvularia* sp., *Dactylaria*-like, *Fusarium*-like, *Nigrospora* sp. *Passalora* sp. and two *Pseudocercospora* spp.). Observations of the damage caused by such fungal diseases in the field indicate that the fungi with the best potential as biological agents are *C. ipomoeae*, *P. puta*, *Albugo* sp., the *Phyllostica* sp. that colonizes stems, and *Phomopsis* sp.

Keywords: aquatic weeds, biological control, coevolved pathogens, *Ipomoea fistulosa*, *Ipomoea carnea* subsp. *fistulosa.*

Introduction

Morning glory, *Ipomoea carnea* Jacq., (local name in Brazil is algodão-bravo) is a shrubby perennial amphibious plant belonging to the Convolvulaceae. It is considered to be native to South America and particularly common in the basins of the rivers Paraguay and São Francisco (Lorenzi, 2000). It is also widely distributed in Brazil as an ornamental species for its showy violet flowers (Kissmann and Groth, 1995). This plant is also one of the most feared poisonous weeds to Brazilian ranchers since it is able to cause severe nervous disorder when ingested by bovines, sheep or goats (Tokarnia *et al*., 2000).

Ipomoea carnea was introduced into areas outside the Neotropics, and it now causes serious invasions of wetland habitats in Southern India and Pakistan where streams, mangroves and other ecosystems may be blocked, hampering irrigation and access (H.C. Evans personal communication, 2006). It is also included in the Florida Exotic Pest Council's List of Florida's Most Invasive Species as a weed category II (FLEPPC, 2003).

Surveys of fungal pathogens of plants native to Brazil that are weeds elsewhere have yielded a plethora of potential biocontrol agents (Barreto and Evans, 1994, 1995a,b, 1998; Barreto and Torres, 1999; Barreto *et al*., 1995, 1999a,b, 2000; Pereira and Barreto, 2000, 2005; Monteiro *et al*., 2003; Soares and Barreto, 2006; Soares *et al*., 2006), and two of the fungi highlighted as promising classical biocontrol agents during such surveys have already been introduced from Brazil into other regions of the world: *Prospodium tuberculatum* (Speg.) Arthur for the biological control of *Lantana camara* L. (Ellison *et al*., 2006) in Australia and *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. for the biological control of *Miconia calvescens* DC in Hawaii (Barreto *et al*., 2001).

Recently, a survey for fungal pathogens of *I. carnea* was started, aimed at finding fungi to be used in the future as biocontrol agents for this weed. *Puccinia puta* H. S. Jacks. and Holw. ex F. Kern, Thurst. and Whetzel

[1] Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil.
Corresponding author: D.J. Soares <dartjs@yahoo.com.br>.

was the only fungus previously recorded on *I. carnea* (referred to as *Ipomoea crassicaulis*) in Brazil. Recently, a second rust fungus, *Coleosporium ipomoeae* (Schwein.) Burril, was recorded by Vieira *et al*., (2004).

Materials and methods

The collecting procedure adopted during the survey was as described in Barreto (1991). The collecting trips occurred between July 2004 and February 2006. Information on some ad hoc collections that were made before the main survey work is also included. The survey covered a wide geographic area of central and southern Brazil including the states of Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo, Paraná, Santa Catarina, Rio Grande do Sul, Mato Grosso, Mato Grosso do Sul, Goiás and Rondônia.

The diseased parts of the plants suspected to be damaged by fungal pathogens were collected, dried in a plant press and taken to the lab. The isolation of the potential agents was performed by direct transfer of fungal structures to Petri dishes containing 15 ml of VBA medium (Pereira *et al.,* 2003), with the help of a dissecting microscope and a sterilized fine point needle. The fungi obtained were preserved in silica-gel according to Dhingra and Sinclair (1996).

Selected specimens were deposited in the local herbarium (Herbarium VIC). Fungal structures were removed from diseased tissues and mounted in lactophenol. Observations of morphology were carried out with an OLYMPUS BX 50 light microscope.

In order to confirm the pathogenicity of two selected fungi (*Passalora* sp. and *Alternaria* sp.), isolates were cultivated in VBA and incubated in the dark for 48 h at 25°C and later submitted to 12 h near-ultraviolet irradiation and 12 h dark. Four disks taken from 10-day-old cultures were placed abaxially and adaxially on three leaves of two healthy potted *I. carnea* plants. After inoculation, plants were left for 48 h in a humid chamber prepared by covering the plants with plastic

Table 1. Fungi recorded on *Ipomoea carnea* from Brazil by Soares (2007).

Fungus	Disease	Damage to host	Purported specificity	Culturability	Biocontrol potential
Aecidium cf. *distinguendum*	Rust	Significant	To the genus *Ipomoea*	Not cultivable	High
Albugo sp.	White rust	Significant	High	Not cultivable	High
Alternaria alternata	Leaf-spot	Significant	Non-specific	Cultivable	Uncertain
Cercospora sp.	Leaf-spot	Insignificant	Not investigated	Cultivable	Low
Cladosporium sp.	Associated to leaf-spots	Insignificant	Low	Cultivable	Low
Coleosporium ipomoeae	Rust	Significant	To the genus *Ipomoea*	Not cultivable	High
Colletotrichum sp.	Anthracnose (stems)	Moderate	Uncertain	Cultivable	Moderate
Curvularia sp.	Associated to leaf-spots	Insignificant	Low	Cultivable	None
Dactylaria-like	Associated to leaf-spots	Insignificant	Uncertain	Cultivable	None
Fusarium-like	Associated to leaf-spots	Insignificant	Uncertain	Cultivable	None
Mycosphaerella sp.	Leaf-spot	Moderate	High	Cultivable	Moderate
Nigrospora sp.	Associated to leaf-spots	Insignificant	Low	Cultivable	None
Passalora sp.	Leaf-spot	Significant	High	Cultivable	High
Phoma sp.	Associated to leaf-spots	Insignificant	Low	Cultivable	Low
Phomopsis sp.	Stem necrosis	Significant	Uncertain	Cultivable	High
Phyllosticta sp. 1	Stem and petiole blight	Severe	High	Apparently not cultivable	Very high
Phyllosticta sp. 2	Associated to leaf-spots	Insignificant	Uncertain	Cultivable	Low
Pseudocercospora sp. 1	Leaf-spot	Moderate	High	Cultivable	Moderate
Pseudocercospora sp. 2	Leaf-spot	Moderate	High	Cultivable	Moderate
Puccinia puta	Rust	Significant	To the genus *Ipomoea*	Not cultivable	High
Unidentified Ascomycete	Stem canker	Significant	Uncertain	Attempts unsuccessful	High

bags wetted inside and having water-soaked cotton internally and left at room temperature (approximately 25°C). After that period, the plastic bags were removed, and plants were left on a bench under room conditions and observed daily for the appearance of symptoms. Three non-inoculated leaves of each of two healthy plants, kept under the same conditions, served as controls.

Results

Twenty-one fungal species were found in association with *I. carnea* during the survey (Table 1). Among these, at least two taxonomic novelties were promptly recognized and will be dealt with separately in a taxonomic publication, namely: *Passalora* sp. and *Phyllosticta* sp.1. All the other fungi that were found represented new host or geographic records.

Inoculation of *I. carnea* with *Passalora* sp. yielded symptoms equivalent to those observed in the field on all inoculated leaves after 20 days. Non-inoculated leaves remained healthy. Typical structures of the *Passalora* sp. were present on the diseased tissues, and the fungus was re-isolated from newly infected tissues.

The species of *Alternaria* on *I. carnea* had the morphology and cultural characteristics typical of *Alternaria alternata* (Fr.) Keissler. Its pathogenicity to *I. carnea* was proven, and similar symptoms to those observed in the field were observed within 15 days of inoculation. This fungus has not been recorded on *I. carnea* until now.

Attempts to isolate the *Phyllostica* sp.1 associated with stem and petiole lesions were unsuccessful. This fungus appears to have a biotrophic habit. Plant tissues surrounding the fungus colonies were observed to retain a healthy appearance until late stages of infection. Necrosis, leaf drop and death of the apical buds only occurred at the final stages of infection.

Discussion

At the present stage of this research, it would be too early to dismiss any of the fungi as not promising for use as biocontrol agents for *I. carnea*. Some of the fungi collected in association with *I. carnea* are either evident saprophytes or suspected to have such status, as is the case of *Nigrospora* sp., *Cladosporium* sp., *Curvularia* sp., the *Dactylaria*-like fungus, the *Fusarium*-like fungus and *Phyllosticta* sp.2. Otherwise, the damage associated with the other fungi, listed in Table 1, was significant as observed in the field. In general, plants infected with such fungi appeared weaker and defoliated as compared with individuals in healthy *I. carnea* populations. The fungi appearing to be the most promising candidates for use in weed biocontrol, deserving further evaluations are: the rusts *C. ipomoeae* and *P. puta*, *Albugo* sp., *Passalora* sp., *Phyllostica* sp.1 and *Phomopsis* sp.

Both rust fungi were frequently found throughout the year associated with moderately high plant defoliation. However, they appear to have a wide host range within the Convolvulaceae since both have been recorded on other species in this family, including sweet potato (in the case of *C. ipomoeae*; Hennen *et al*., 2005). There may be host-specific strains of *C. ipomoeae* and *P. puta* that could safely be introduced into other regions of the globe, but even if these species are proven to be polyphagous within the Convolvulaceae, their introduction into other areas of Brazil against noxious *I. carnea* population might still be considered.

Albugo sp. was found only a few times, in the states of Mato Grosso, Mato Grosso do Sul and São Paulo. This fungus appears to have a more restricted geographic distribution compared with the two rusts. It caused a complete leaf curling or leaf blight (when the attack occurred on the petioles). However, its specificity and potential to be used as a biocontrol agent requires further investigation.

Passalora sp. could prove useful as a classical biological control or even as a mycoherbicide against *I. carnea*. Although no sporulation was obtained for this fungus in the conditions that were used, the potential for mass production of spores, which is critical for its viability as a mycoherbicide, was not properly investigated.

Phomopsis sp. was consistently found associated with stem necrosis and easily sporuled in culture; however, its pathogenicty and specificity has not yet been tested.

Phyllosticta sp.1 appears to be the most promising candidate to be used as a classical biological control agent. The damage inflicted naturally by this fungus on *I. carnea* populations was evident. Infected plants in advanced disease stages were weakened and almost completely defoliated. On diseased plants, foliage on each individual stem was often reduced to only six or eight terminal leaves.

Although it used to be thought that *A. alternata* had several pathotypes that produce host-specific toxins, this was considered wrong by Simmons (1999). If further investigation on this fungus on *I. carnea* confirms that it fits within the non-specific, cosmopolitan *A. alternata*-group, this would restrict its potential as a classical biocontrol agent but not necessarily result in its rejection for use as a biocontrol agent of *I. carnea.* This fungus grows well and sporulates abundantly in culture and could be further evaluated for development of a mycoherbicide to be used in Brazil similarly to what is being done with an isolate of *A. alternata* obtained from *Eichhornia crassipes* in India (Babu *et al*., 2002, 2003, 2004).

Half of the fungi previously recorded in the literature in association with *I. carnea* were recorded only from countries outside the native range of this plant species in the Neotropics. Most of the records from countries such as India, Pakistan and Malaysia probably represent saprophytic, weakly pathogenic–opportunistic or generalistic pathogens of no relevance for biocontrol (Table 2).

Table 2. Fungi recorded on *Ipomoea carnea* and their synonyms worldwide. Extracted from Farr *et al*. (no date).

Fungus name	Country/Region
Aecidium agnesiae (Syd.) Z. Urb.	Cuba
Aecidium distinguendum P. Syd. and Syd.	Caribbean; Cuba; Venezuela
Aecidium sp.	Venezuela
Albugo ipomoeae (as spelt by the author)	Cuba
Albugo ipomoeae-panduratae (Schwein.) Swingle	Caribbean; Cuba
Aplosporella ipomoeae S. Ahmad	India; Pakistan
Botryodiplodia theobromae Pat.	Pakistan
Capnodium sp.	Caribbean; Cuba
Cercospora ipomoeae G. Winter	India
Coleosporium ipomoeae	Cuba; Colombia; Brazil
Cytospora ipomoeae S. Ahmad and Arshad	India
Dischloridium cylindrospermum S.K. Srivast.	India
Dothiorella ipomoeae S. Ahmad	India
Guignardia cytisi (Fuckel) Arx and E. Müll.	Pakistan
Lasiodiplodia theobromae (Pat.) Griffon and Maubl.	Venezuela
Leptosphaeria macrospora (Fuckel) Thüm.	Pakistan
Macrophoma ipomoeae Pass.;	India; Pakistan
Marasmiellus scandens (Massee) Dennis and D.A. Reid	Malaysia
Meliola malacotricha Speg.	Malaysia
Monilochaetes infuscans Harter	India
Munkovalsaria donacina (Niessl) Aptroot	India
Ophiobolus herpotrichus (Fr.) Sacc.	Pakistan
Periconia byssoides Pers.	Venezuela
Pestalotiopsis adusta (Ellis and Everh.) Steyaert	India
Phoma herbarum var. *herbarum* Westend.	Pakistan
Phomopsis ipomoeae Petr.	Venezuela
Pseudocercosporella ipomoeae Sawada ex Deighton	Venezuela
Puccinia achyroclines (Henn.) H.S. Jacks. and Holw.[a]	Brazil
Puccinia distinguenda H.S. Jacks. and Holw.	Ecuador; Venezuela
Puccinia megalospora (Orton) Arthur and J.R. Johnst.	Mexico
Puccinia nocticolor Holw.	Guatemala
Puccinia puta	Colombia; Venezuela; Puerto Rico; Brazil
Puccinia rubicunda Holw.	Mexico
Tuberculina persicina (Ditmar) Sacc.	Caribbean

[a] This record is regarded here as dubious, since the original publication (Hennen *et al*., 1982) which was cited by Farr *et al*. (no date) makes no mention of *I. carnea* or its synonyms as host for this fungus.

Although only a relatively limited area of the native range of *I. carnea* was surveyed, several potential biocontrol agents were found. It is, therefore, expected that the expansion of the survey into new areas in Brazil or other parts of the Neotropics will reveal a much larger list of potential fungal agents for biocontrol of *I. carnea*. Although Brazil is considered as part of the centre of origin of this plant, only two fungi were previously known on this host in Brazil. Results of the present study added 19 new taxa to this list, most of which are clearly pathogenic to *I. carnea* (Table 1). Pathogenicity and host-specific tests are now being conducted to confirm the status of fungi selected as having possible potential for use in the biocontrol of *I. carnea.*

Acknowledgements

This work forms part of a research project submitted as a PhD dissertation to the Departamento de Fitopatologia/Universidade Federal de Viçosa by D.J. Soares. The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

References

Babu, R.M., Sajeena, A., Seetharaman K., Vidhyasekaran, P., Rangasamy, P., Prakash, M.S., Raja, A.S. and Biji, K.R. (2002) Host range of *Alternaria alternata* - a potential fungal biocontrol agent for waterhyacinth in India. *Crop Protection* 21, 1083–1085.

Babu, R.M., Sajeena, A. and Seetharaman K. (2003) Bioassay of the potentiality of *Alternaria alternata* (Fr.) Keissler as a bioherbicide to control waterhyacinth and other aquatic weeds. *Crop Protection* 22, 1005–1013.

Babu, R.M., Sajeena, A. and Seetharaman K. (2004) Solid substrate for production of *Alternaria alternata* conidia:

a potential mycoherbicide for the control of *Eichhornia crassipes* (waterhyacinth). *Weed Research* 44, 298–304.

Barreto, R.W. (1991) *Studies on the pathogenic mycoflora of selected weeds from the State of Rio de Janeiro (Brazil)*, PhD thesis, University of Reading, England, UK.

Barreto, R.W. and Evans, H.C. (1994) The mycobiota of the weed *Chromolaena odorata* in southern Brazil with particular reference to fungal pathogens for biological control. *Mycological Research* 98, 1107–1116.

Barreto, R.W. and Evans, H.C. (1995a) Mycobiota of the weed *Cyperus rotundus* in the state of Rio de Janeiro, with an elucidation of its associated *Puccinia* complex. *Mycological Research* 99, 407–419.

Barreto, R.W. and Evans, H.C. (1995b) The mycobiota of the weed *Mikania micrantha* in southern Brazil with particular reference to fungal pathogens for biological control. *Mycological Research* 99, 343–352.

Barreto, R.W. and Evans, H.C. (1998) Fungal pathogens of *Euphorbia heterophylla* and *E. hirta* in Brazil and their potential as weed biocontrol agents. *Mycopathologia* 141, 21–36.

Barreto, R.W. and Torres, A.N.L. (1999) *Nimbya alternantherae and Cercospora alternantherae*: two new records of fungal pathogens on *Alternanthera philoxeroides* (alligatorweed) in Brazil. *Australasian Plant Pathology* 28, 103–107.

Barreto, R.W., Evans, H.C. and Ellison, C.A. (1995) The mycobiota of the weed *Lantana camara* in Brazil, with particular reference to biological control. *Mycological Research* 99, 769–782.

Barreto, R.W., Evans, H.C. and Hanada, R.E. (1999a) First record of *Cercospora pistiae* causing leaf spot of water lettuce (*Pistia stratiotes*) in Brazil, with particular reference to weed biocontrol. *Mycopathologia* 144, 81–85.

Barreto, R.W., Evans, H.C. and Pomella, A.W.V. (1999b) Fungal pathogens of *Calotropis procera* (rubber bush), with two new records from Brazil. *Australasian Plant Pathology* 28, 126–130.

Barreto, R.W., Charudattan, R., Pomella, A. and Hanada, R. (2000) Biological control of neotropical aquatic weeds with fungi. *Crop Protection* 19, 697–703.

Barreto, R.W., Seixas, C.D.S. and Killgore, E. (2001) *Colletotrichum gloeosporioides* f.sp. *miconiae*: o primeiro fungo fitopatogênico brasileiro a ser introduzido no exterior para o controle biológico clássico de uma planta invasoras (*Miconia calvescens*) In: *7th Simpósio de Controle Biológico*. 03–07 June 2001, Poços de Caldas.

Dhingra, O.D. and Sinclair, J.B. (1996) *Basic Plant Pathology Methods*, 2nd ed. Lewis Publishers, Boca Raton, FL.

Ellison, C.A., Pereira, J.M., Thomas, S.E., Barreto, R.W. and Evans, H.C. (2006) Studies on the rust *Prospodium tuberculatum*, a new classical biological control agent released against the invasive weed *Lantana camara* in Australia. 1. Life-cycle and infection parameters. *Australasian Plant Pathology* 35, 309–319.

Farr, D.F., Rossman, A.Y., Palm, M.E., and McCray, E.B. (no date) *Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA*. Available at: http://nt.ars-grin.gov/fungaldatabases/, accessed March 21, 2007.

FLEPPC (2003) *List of Florida's Invasive Species. Florida Exotic Pest Plant Council*. Available at: http://www.fleppc.org/03list.htm.

Hennen, J.F., Figueiredo, M.B., Caralho Jr, A.A. and Hennen, P.G. (2005) *Catalogue of the species of plant rust fungi (Uredinales) of Brazil*. Available at: http://www.jbrj.gov.br/publica/uredinales/Brazil_Catalogue1drevisado.pdf.

Hennen, J.F., Hennen, M.M. and Figueiredo, M.B. (1982) Índice das ferrugens (Uredinales) do Brasil. *Arquivos do Instituto Biológico de São Paulo* 49(Suppl. 1), 1–201

Kissmann, K.G. and Groth, D. (1995) *Plantas Infestantes e Nocivas*. Tomo II. BASF S.A., São Paulo, Brasil.

Lorenzi, R. (2000) *Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas.* Instituto Plantarum de Estudos da Flora Ltda. Nova Odessa, Brasil.

Monteiro, F.T., Vieira, B.S. and Barreto, R.W. (2003) *Curvularia lunata and Phyllachora* sp.: two fungal pathogens of the grassy weed *Hymenachne amplexicaulis* from Brazil. *Australasian Plant Pathology* 32, 449–453.

Pereira, J.M. and Barreto, R.W. (2000) Additions to the mycobiota of the weed *Lantana camara* (Verbenaceae) in southeastern Brazil. *Mycopathologia* 151, 71–80.

Pereira, J.M., Barreto, R.W., Ellison, A.C. and Mafia, L.A. (2003) *Corynespora casiicola* f. sp. *lantanae*: a potential biocontrol agent from Brazil for *Lantana camara*. *Biological Control* 26, 21–31.

Pereira, O.L. and Barreto, R.W. (2005) The mycobiota of the weed *Mitracarpus hirtus* in Minas Gerais (Brazil) with particular reference to fungal pathogens for biological control. *Australasian Plant Pathology* 34, 41–50.

Simmons, E.G. (1999) *Alternaria* themes and variations (236–243) Host-specific toxin producers. *Mycotaxon* 70, 325–369.

Soares, D.J. (2007) *Fungos associados à once plantas aquáticas no Brasil e seu potencial para controle biológico*. PhD thesis. Universidade Federal de Viçosa, MG/Brasil.

Soares, D.J. and Barreto, R.W. (2006) Additions to the Brazilian mycobiota of the grassy weed, *Hymenachne amplexicaulis*, with a discussion on the taxonomic status of *Paraphaeosphaeria recurvifoliae*. *Australasian Plant Pathology* 35, 347–353.

Soares, D.J. Ferreira, F.A. and Barreto, R.W. (2006) First report of the aecial stage of *Puccinia scirpi* on *Nymphoides indica* in Brazil, with comments on its worldwide distribution. *Australasian Plant Pathology* 35, 81–84.

Tokarnia, C.H., Döbereiner, J. and Peixoto, P.V. (2000) *Plantas tóxicas do Brasil*. Editora Helianthus, Rio de Janeiro, Brazil.

Vieira, F.M.C., Pereira, O.L. and Barreto, R.W. (2004) First report of C*oleosporium ipomoeae* on *Ipomoea fistulosa* in Brazil. *Fitopatologia Brasileira* 29, 693.