Fungal latent pathogens and endophytes from leaves of *Parthenium hysterophorus* (*Asteraceae*)

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Endophytic fungi were isolated from leaves of the weed *Parthenium hysterophorus* in order to establish whether the endophytes were the same fungi as had previously been recorded on scenescent or diseased leaf tissues. Seven surface sterilization methods were used. *Alternaria zinniae*, *A. helianthi*, *Cylindrocarpon* sp., *Curvularia brachyspora*, *Fusarium* sp., *Nigrospora oryzae*, *Penicillium funiculosum* and *Periconia* sp. were isolated The methods used to isolate endophytes may represent a tool for the identification of biological control agents of weeds.

Key words: biological control, endophytes, weeds.

Introduction

Parthenium hysterophorus (*Asteraceae*: *Heliantheae*) is a native herbaceous plant from tropical and subtropical America (Nash and William, 1976). In Australia, India and Madagascar, it has invaded urban and agriculture zones and has become a weed. The plant also causes breathing problems, dermatitis, and allergies, in susceptible people and animals (Towers *et al.*, 1992; Dhawan *et al.*, 1993). Insects and fungal pathogens from northeast Mexico have been assessed for their potential in the biological control of *P. hysterophorus* (Mcfayden, 1985; Evans 1987; Parker *et al.*, 1994; McClay *et al.*, 1995). In standard classical biological control, obligate parasites, in this case rust fungi (Uredinales) are the first choice, because they exhibit narrow host ranges, high reproductive capacities, and fast, efficient aerial dispersal (Evans and Ellison, 1990). In the case of *Parthenium* the most promising fungal agents are: *Puccinia abrupta* var. *parthenicola* (Jackson) Parmelee (Uredinales), *P. melampodii* Diet. and Holw. (Uredinales), *Plasmopara halstedii* (Farlow) Berl. and De Toni (Peronosporales), and *Entyloma compositarum* De Bary (Ustilaginales). Facultative pathogens of *P. hysterophorus* may also be appropiate fungal pathogens for biological control and could be mass-produced in the laboratory for future application in the field.

Seier and Romero (1996, 1997) carried out surveys in various regions of Mexico, in order to determine the fungal pathogens that could be useful as biocontrol agents of this weed, and collected nine mitosporic fungi associated with chlorotic and necrotic symptoms on old leaf tissues (Table 1). When a potential agent is sought for use in the biological control of a target weed, symptomatology helps in the selection process. Unspecialized pathogenic, mutualistic or endophytic fungi can however, produce symptoms similar to those of potential biological control agents.

Stone *et al.* (1994) considered that endophytes have common attributes: 1) they are internal, at least subticular, and have contact with and derive nutrition from the living host tissue; 2) they establish at least a transitory biotrophic nutritional relationship with their host; and 3) infected host tissues remain symptomless, i.e. disease free during their lifetime. Endophytes are also thought to be latent pathogens (Brown *et al.*, 1998). Asymptomatic endophytes have diverse relationships with their host plants and may only produce disease symptoms under certain conditions. The purpose of this study is to identify endophyte species that may exist as latent pathogens in *P. hysterophorus*, and evaluate methods to isolate these fungi from asymptomatic hosts.

Materials and methods

Endophytes were isolated from leaves of mature healthy plants of *Parthenium hysterophorus*, from three sites in the central region of the state of Veracruz: Cotaxtla, Misantla and Emiliano Zapata. The vegetation of Cotaxtla was originally tropical rainforest, but is now mostly mango plantations (*Mangifera indica* L.). Misantla was also a tropical forest, but has now been converted to grassland. Emiliano Zapata was a mixture of tropical deciduous forest and oak forest (*Quercus* spp.) and currently it is an agricultural area where vegetables are cultivated. *P. hysterophorus* is part of the native flora at all sites.

Isolations were carried out in June and November 1996. Leaf surfaces were washed with distilled and sterilized water in order to remove epiphytic fungi. Seven methods of surface sterilisation (Schulz *et al.*, 1993: Table 2) were utilised. Fifteen segments (5-10 mm^2) were cut from leaves and used for each method, 105 segments were sterilized form each location and collection period. To stimulate the development of the mycelia, leaf segments were placed in Petri dishes with agar and malt extract (3.36%, Bioxon) and incubated at room temperature (~ 21 C). The mycelium developed within two weeks and the fungi were then transfered to new Petri dishes with the same media. The fungi were identified following sporulation.

Results

One-hundred and twenty-five endophyte strains were isolated from 420 leaf segments. Twenty-five "mycelia sterilia" (*sensu* Taylor *et al.*, 1999) never sporulated, and eight had yeast-like growth (Table 3). *Alternaria helianthi* (Hansford) Tubaki and Nishihara was isolated from all sites. *Alternaria alternata* (Fr.) Keissler, *Fusarium* sp. and *Penicillium funiculosum* Thom. were isolated from two sites. *Nigrospora oryzae* (Berk and Broome) Petch and *Periconia* sp. were isolated from Site 1, *Curvularia brachyspora* Bredijn from Site 2, and, *Cylindrocarpon* sp. from Site 3 (Table 3). *Fusarium* sp. and *Nigrospora oryzae* have note been recorded previously from this host (Table 1).

Table 1. Fungi previously recorded as pathogens from leaves of *Parthenium hysterophorus* in the state of Veracruz, Mexico (Seier and Romero, 1996, 1997). *Species also isolated as endophytic fungi.

Fungus
**Alternaria alternata* (Fr.) Keissler
**A. helianthi* (Hansford) Tubaki and Nishihara
A. protenta Simmons
A. zinniae M.B. Ellis
Cercospora sp.
**Cylindrocarpon* sp.
**Penicillium funiculosum* Thom.
**Periconia* sp.
Phoma subglomerata Boerema

Surface sterilization method I, yielded the largest number of endophytes (73 strains). However, it is possible that some epiphytes may also have been isolated with this method. Method III revealed the second largest number of endophytes (34 strains). Methods II and IV yielded 15 and three strains, respectively. In the case of methods V, VI and VII, where formaldhyde was used, no development of mycelium was observed.

Pathogens previously recorded from leaves of *Parthenium hysterophorus* are listed in the Table 1. Strains of five of the nine species live endophytically in tissues of *P. hysterophorus* (Table 2).

Discussion

Most mitosporic fungi isolated as endophytes in this study also grow and sporulate on chlorotic and necrotic leaf tissues of *P. hysterophorus*. As some pathogens may have a latent phase within the host tissue, and some saprobes can also be facultative parasites, it may be that certain endophytes become

Table 2. Sterilization surface methods used with leaves of *Parthenium hysterophorus* (based on Schulz *et al.*, 1993).

Method	Solution	Dilution with distilled water	Time in solution
I	NaOCl (15%)	2:1	5 min
II	Ethanol 96%	2:1	1 min
	NaOCl		5 min
	Ethanol 96%		30 sec
III	Ethanol 96%	1:3	30 sec
	Sterile water		30 sec
	NaOCl		5 min
	Ethanol 96%		30 sec
	Sterile water		2 min
IV	Ethanol 96%		5 min
V	Formaldehyde 40%		1 min
VI	Formaldehyde 40%		3 min
VII	Formaldehyde 40%		5 min

pathogenic when the host plant is stressed. Evidence suggests that endophytes have evolved directly from plant pathogenic fungi (Carrol, 1998; Isaac, 1992). A latent phase represents a specific condition where the fungus can either develop symptoms or cause changes in the physiology of the host plant. This condition may not be suitable in the biological control programme of weeds, because these fungi may rarely become pathogenic if the plant is healthy. Fungal biocontrol agents should have a rapid life cycles and cause damage to the host plants (Evans and Ellison, 1990). In other cases however, endophytic relationships represent an advantage in the control of pest and diseases in economically important plants or grasses (Clay, 1988; 1989). The host-endophyte association may result in protection against insects or other pathogens, instead of causing damage. Welty *et al.* (1991) observed that in the grass *Festuca arundinacea* Schreb the presence of endophytes reduce the incidence of *Puccinia graminis* subsp. *graminicola* Z. Urban and *Rhizoctonia zeae* Voorhees (Gwinn and Gavin, 1992). Schardl and Phillips (1997) reported *Epichloë* and *Neotyphodium* species protecting various grasses against fungal pathogens, nematodes, insect and mammalian herbivores. It has been suggested that some endophytic species may be antagonistic towards other fungal species (Isaac, 1992). Because endophytes colonize the internal tissues of the host plant. This strategy assures the early occupation and possession of the resources, before the colonization by other fungi can occur, besides the production of alkaloids, terpenes and others, during symbiont stage. If this is the case the presence of endophytes in host plant may be problematical in

biological control, because potential biological agents may be inhibited by the endophytes.

Table 3. Endophytic fungi isolated from leaves of *Parthenium hysterophorus* from three sites in the state of Veracruz, México. Methods are the same as in Table 2; [1]previously identified as pathogenic fungi (see Table 1); ni = not isolated in this month; F = frequency.

Site	Endophytes	Isolation method			
		June	F (%)	Nov	F (%)
Cotaxtla	[1]*Alternaria helianthi*	I	12	I	9
	[1]*A. alternata*	IV	4	ni	ni
	Fusarium sp.	II	18	I	11
	Nigrospora oryzae	ni	ni	I	5
	[1]*Penicillium funiculosum*	III	6	ni	ni
	[1]*Periconia* sp.	I	6	ni	ni
	Unidentified sp. 1	III	12	ni	ni
Misantla	[1]*A. helianthi*	I	9	I	7
	Curvularia brachyspora	ni	ni	I	5
	Fusarium sp.	I	8	I	10
	[1]*Penicillium funiculosum*	I	4	ni	ni
	Unidentified sp. 2	ni	ni	III	4
	Unidentified sp. 3	ni	ni	III	5
	Yeast sp. 1	ni	ni	III	7
	Yeast sp. 2	ni	ni	III	5
Emiliano Zapata	[1]*A. helianthi*	I	14	I	11
	[1]*A. alternata*	I	4	ni	ni
	[1]*Cylindrocarpon* sp.	I	3	ni	ni
	Unidentified sp. 4	ni	ni	II	5
	Unidentified sp. 5	ni	ni	III	9
	Unidentified sp. 6	ni	ni	III	7

Our comparison of the surface sterilization methods, shows that methods I and III are most effective. With Method I we isolated most endophyte strains that produce spores. Method III, is probably used most often by reserchers (Petrini *et al.*, 1992), and was more effective in isolating sterile mycelium and yeasts. Both methods can be used to obtain endophytic fungi in other herbaceus plants. Methods II and IV were more or less specific to *Fusarium* sp. and *A. alternata*, but their frequency was low. Methods V, VI and VII seem to destroy the endophytes within the plant tissue. It is possible that these methods could be more useful if we reduced concentration and/or times of exposure to solutions. Schulz *et al.* (1993) obtained best results using method IV, in eleven of the twelve species of plants tested. Leaf tissue characteristics vary with each plant species, thus several isolation methods should be tested in order to determine the optimum isolation protocol. There is however, a risk in isolating

pathogenic strains, either when the pathogen is in an incubation phase or it is latent within the host tissue. It is also possible that some of the fungi isolated as endophytes, are epiphytes.

An understanding of endophytes present in the target weed should prove to be valuable in biological control regimes. This may increase the possibility of success of the agents selected. If endophytic relationships of potential biocontrol agents are recognized within host plants, or if the establishment of biocontrol agents are affected by endophytes inside leaf tissues, some pathogenic fungi may have to be discarded as potential biocontrol agents.

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