LIDIANE LEAL DUARTE

MICOBIOTA ASSOCIADA À PLANTA DANINHA *Conyza canadensis* NO BRASIL COM PARTICULAR REFERÊNCIA AOS FUNGOS FITOPATOGÊNICOS PARA O CONTROLE BIOLÓGICO

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título *Magister Scientiae*.

VIÇOSA
MINAS GERAIS - BRASIL
2011

Ficha catalográfica preparada pela Seção de Catalogação e Classificação da Biblioteca Central da UFV

T
D812m
2011

Duarte, Lidiane Leal, 1985-
Micobiota associada à planta daninha *Conyza canadensis* no Brasil com particular referência aos fungos fitopatogênicos para o controle biológico / Lidiane Leal Duarte. – Viçosa, MG, 2011.
ix, 75f. : il. (algumas col.) ; 29cm.

Orientador: Robert Weingart Barreto.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f. 42-47

1. Fungos - Identificação. 2. Fungos fitopatogênicos. 3. *Conyza canadensis*. 4. *Conyza canadensis* - Controle biológico. 5. Ervas daninhas - Controle. I. Universidade Federal de Viçosa. II. Título.

CDD 22. ed. 632.4

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APROVADA: 14 de julho de 2011

Dr. Dartanha José Soares

Prof. Leandro Grassi de Freitas

Prof. Ludwig Heinrich Pfenning

Prof. Olinto Liparini Pereira
(Co-orientador)

Prof. Robert Weingart Barreto
(Orientador)

A Deus;
Aos meus pais, Mauricio e Ângela;
As minhas irmãs, Cristiane e Josiane;
Ao Eduardo.

Por serem o meu apoio.

Dedico!

"The mind, once expanded to the dimensions of larger ideas, never returns to its original size."
Oliver W. Holmes

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AGRADECIMENTOS

A Deus, por ser o meu refúgio e minha fortaleza.

Aos meus pais, pela constante dedicação, renúncia, amor e confiança. Por acreditarem e incentivarem os meus sonhos.

As minhas irmãs, pela amizade e carinho que demostraram ao longo dessa caminhada.

Ao meu namorado e melhor amigo Eduardo, pelo imenso apoio e presença em todos os momentos, pela paciência nas horas mais difícies e por nunca me deixar desanimar. Agradeço acima de tudo o seu amor e a torcida que me faz sempre mais forte e me incentiva a seguir em frente.

Ao Pe. Geraldo, pelas orações.

Ao Prof. Robert Weingart Barreto, pela orientação, ensinamentos, paciência e por ter acreditado que eu era capaz.

Ao Prof. Olinto Liparini Pereira e ao Dr. Harry Charles Evans, pelas sugestões e atenção dispensada.

Aos professores do Departamento de Fitopatologia, por terem contribuido na construção de mais um grau no meu conhecimento.

A minha estagiária Fabiana, por ter me auxiliado na preparação desse trabalho.

Aos colegas da Clínica de Doenças de Plantas, pelo divertido convívio e amizade.

Aos funcionários do Departamento de Fitopatologia e da Clínica de Doenças de Plantas, pelos serviços prestados e pela gentileza e educação com que sempre me trataram.

A CAPES, pela concessão da bolsa de estudos.

A todos que direta ou indiretamente contribuíram para a realização desse trabalho.

Muito obrigada!

BIOGRAFIA

LIDIANE LEAL DUARTE, filha de Maurício Lopes Duarte e Ângela Maria das Dôres Leal Duarte, nasceu na cidade de Viçosa-MG, no dia 23 de outubro de 1985.

Realizou todos os estudos básicos na cidade de Conselheiro Lafaiete-MG.

Em 2004, iniciou o curso de graduação em Agronomia na Universidade Federal de Viçosa, graduando-se em janeiro de 2009.

Em julho de 2009, iniciou o programa de mestrado em Fitopatologia na Universidade Federal de Viçosa, concentrando seus estudos na área de Micologia (Taxonomia de fungos fitopatogênicos).

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RESUMO

DUARTE, Lidiane Leal, M.Sc., Universidade Federal de Viçosa, julho de 2011. **Micobiota associada à planta daninha *Conyza canadensis* no Brasil com particular referência aos fungos fitopatogênicos para o controle biológico.** Orientador: Robert Weingart Barreto. Co-orientador: Olinto Liparini Pereira.

Conyza canadensis (Asteraceae), popularmente conhecida no Brasil como buva, é uma planta anual ou bienal nativa da América, possivelmente da América do Norte. Trata-se de uma espécie muito prolífica, podendo produzir até 200.000 sementes viáveis por planta, que se adapta bem a ambientes alterados pelo homem sob diversas condições climáticas, tem boa dispersão de sementes pelo vento e ainda inclui populações com resistência a vários tipos de herbicida. Essa combinação de atributos tem feito com que ela se tornasse um sério problema em diversas lavouras no Brasil e pelo mundo. No Brasil, os maiores problemas relativos a essa daninha se concentram em pomares de citrus e nas áreas produtoras de soja, principalmente em cultivos de soja resistente ao glifosato. Depois da introdução destes cultivares o número de aplicações do herbicida cresceu o que resultou ao longo dos anos, na seleção de biótipos de *C. canadensis* resistentes a este produto. Como conseqüência, houve uma elevação nas falhas de controle, o que tem sido suficiente para limitar a produção em diversas áreas, levando a uma necessidade de se alterar as práticas de manejo e tornando o controle biológico uma ferramenta potencialmente importante para um apropriado manejo da buva. Como ponto de partida para o estudo visando o desenvolvimento de um herbicida biológico para o controle de *C. canadensis* foi realizado um levantamento sistemático dos fungos associados a essa daninha no Brasil visando à descrição da micobiota fitopatogênica e seleção de potenciais agentes de controle biológico. Desse levantamento resultaram 211 amostras, provenientes das regiões sul, sudeste e parte do centro-oeste do Brasil. Foram encontrados, descritos e ilustrados doze fungos, dentre os quais três são aqui reconhecidos como novos taxa. Compreendem esta micobiota: três hifomicetos - *Cercosporella virgaurea*, *Cercospora* sp. nov. e *Alternaria tenuissima*, um oídio- *Podosphaera fusca*; três coelomicetos - *Colletotrichum capsici*, *Phoma canadensis* e *Septoria erigerontis*; três ascomicetos - *Mycosphaerella* sp., *Sphaerulina* sp. nov. e

Wentiomyces melioloides; uma ferrugem - *Aecidium* sp. nov. e um oomiceto - *Basidiophora entospora*. Dentre as espécies encontradas, *Phoma canadensis* parece ter o melhor potencial para o desenvolvimento de um mico-herbicida. Trata-se de fungo de crescimento rápido e esporulação abundante em meio de cultura e capaz de causar a morte da planta hospedeira ou atrasar consideravelmente o seu desenvolvimento do seu hospedeiro. Estudos mais detalhados deverão ser realizados a fim de comprovar a sua viabilidade como mico-herbicida.

ABSTRACT

DUARTE, Lidiane Leal, M.Sc., Universidade Federal de Viçosa, July 2011. **Mycobiota associated with the weed *Conyza canadensis* in Brazil with particular reference to fungal pathogens for biological control.** Adviser: Robert Weingart Barreto. Co-adviser: Olinto Liparini Pereira.

Conyza canadensis (Asteraceae), commonly known as horseweed (common name in Brazil – buva), is an annual or biennial and native to the Americas, possibly from North America. It is well adapted to habitats modified by human activities, very prolific (capable of producing up to 200.000 viable seeds per plant), tolerates a large range of climatic conditions, it is highly effectively dispersed by the wind and includes populations which are naturally resistant to a range of herbicides. Such a combination of features are behind the emergence of *C. canadensis* as a major weed for several crops in Brazil and in other parts of the world. In Brazil, the biggest problems with *C. canadensis* infestations are in citrus and soybean, particularly in transgenic glyphosate-resistant soybean areas. After the use of such soybean cultivars became common place in Brazil, the number of herbicide applications increased which resulted over the years in the selection of herbicide-resistant biotypes of *C. canadensis*. This resulted in increased failure in chemical control and significant losses leading to a need for a change in management practices, including the possible use of biocontrol methods. As the starting point for a study aimed at developing a bioherbicide for *C. canadensis* a broad survey of the existing pathogens (in the present case pathogenic fungi) associated to this weed in Brazil was performed with the purpose of collecting and describing the mycobiota of this weed and selecting a potential biocontrol agents. The survey yielded 211 samples from south and southeatern Brazil which were described and illustrated, three of which are described herein as new to science. This mycobiota includes: trhee hyphomycetes - *Cercosporella virgaureae*, *Cercospora* sp. nov. and *Alternaria tenuissima*, one powdery mildew - *Podosphaera fusca*; three coelomycetes - *Colletotrichum capsici*, *Septoria erigerontis* and *Phoma canadensis*; three ascomycetes - *Mycosphaerella* sp.,

Sphaerulina sp. nov. and *Wentiomyces melioloides*; a rust fungus - *Aecidium* sp. nov. and one oomycetes - *Basidiophora entospora*. Among the species found, *Phoma canadensis* appears to have the greatest biocontrol potential. It is fast-growing and sporulates well in culture, it is capable of causing plant death or significantly delay plant growth. Further studies are necessary for confirmation of its viability as a mycoherbicide.

GENERAL INTRODUCTION

Weed is any plant growing where it is not desired (Singh *et al*., 1996) and does not has any economic value or that competes with man for soil (Cruz, 1979). Weeds are a perpetual menace to agricultural productivity, causing significant reductions in the quantity and quality of crops yields and also pose serious ecological problems for altering ecosystem processes and displacing native plant and animal species (Yandoc-Ables *et al*., 2006). There are no completely accurate estimates of the total cost of weed control and losses in agriculture due to weed competition (Zimdahl, 2007), but it is believed that weeds cause losses worth approximately $150 billion annually, which is equal to about one-third of all crop losses in the world (Agrios, 2005). Only a minority of the world's flora is recognized as having a weed status and among weedy species some are clearly more relevant than others. One genus containing weeds of widely recognized importance is the genus *Conyza* (Asteraceae).

It includes about 50 different species (Kissmann & Groth, 1999) of annual herbs which are mainly adapted to tropical and subtropical areas (Nesom, 1990). *Conyza canadensis* (L.) Cronq, known as horseweed, is the species which has gained the highest importance as a weed in last decades. Holm *et al*. (1997) listed it as a problem weed in more than 40 crops in 70 countries. The center of origin of this weed is thought to be North America from where it has spread to five continents and being regarded as one of the most widely distributed plant species in the word (Frankton & Mulligan, 1987; Thebaud & About, 1995; Heap, 2011).*Conyza* is quoted as representing the most successful case of intercontinental colonization of an American plant in the old world (Thebaud & About, 1995). Lazaroto *et al*. (2008) pointed out that *C. canadensis* broad geographic distribution suggests that it has few climatic limitations.

Conyza canadensis is a ruderal species that often colonizes abandoned agricultural land, roadsides as well as other disturbed habitats (especially pastures, perennial and annual crops) (Thebaud & About, 1995; Weaver, 2001). It is a prolific species, capable of producing up to 200,000 viable seeds per plant which are wind

dispersed (Weaver, 2001), tolerates drought well and produces biomass and seeds under conditions that are stressful for crops (Loux, *et al*., undated). Additionally it includes populations which are resistant to several herbicides (glyphosate, paraquat, triazine and ALS inhibitors) rendering its management in crop situations particularly difficult. It is now ranked among the ten most important herbicide-resistance weeds in the world (Heap, 2011).

In Brazil, the biggest problems with *C. canadensis* infestations are in citrus and soybean plantations, where the yields are reduced due to direct competition for water, nutrients, carbon dioxide and in the case of soybean, competition for light, space and moisture. (Loux, *et al*., undated). In extreme situations of heavy *C. canadensis* infestations the production losses in soybean fields can be of up to 83% (Bruce & Kells, 1990). The repetitive use of herbicides with a single mode of action, based on a single molecule, particularly of glyphosate has favored the emergence of herbicide resistance in *C. canadensis* and rendering the management of this weed increasingly difficult (Guareschi, 2010).

The soybean *Glycine max* (L.) Merrill is the main crop in Brazil in both volume and income generation, being responsible for 1.5% of total PIB, 11% of domestic exports and for the generation of 1,5 million direct and indirect jobs (ABIOVE, 2010). The economic and strategic relevance of soybean as a crop, not only in Brazil but also in a worldwide scale, has encouraged research in the private and public sector towards the development and management of this crop. One significant breakthrough was the release in 1996 of the first commercial transgenic soybean cultivar Roundup Ready soybeans (Duke & Powles, 2009). The mechanism of glyphosate action is the competitive inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) that is responsible for the conversion of phosphoenolpyruvate and shikimate-3-phosphate in inorganic phosphate and EPSP in the shikimic acid pathway. This inhibition results in the accumulation of the shikimic acid and in the reduction in the biosynthesis of aromatic aminoacids (Armhein *et al*., 1980; Geiger & Fuchs, 2002). All the commercial cultivars that are glyphosate resistant have the CP4 transgene, originally found in a soil bacterium *Agrobacterium* sp. - strain CP4, this transgene encodes for an insensitive form of EPSP protein, thus the plants bearing this gene remain unaffected after being sprayed with glyphosate because of the continued action of the

introduced glyphosate tolerant EPSP enzyme which meets the plants need for aromatic amino acids (Padgette *et al*., 1995, 1996).

Glyphosate-resistant transgenic soybean varieties were first officially allowed to be grown in Brazil in the crop season of 2003/2004 and their adoption has been both rapid and substantial (Cerdeira *et al*., 2010). According to Duke & Powles (2009) this rapid adoption was due the facilitation of weed management, cost reduction and ease of use. About 80% of the total area planted with soybeans in Brazil, in the growing year of 2010/2011, corresponded to genetically modified cultivars tolerant to glyphosate and there are other countries where this percentage is even higher (Cerdeira *et al*., 2010; Brookes & Barfood, 2006; Penna & Lema, 2003). In the ongoing season the area planted with soybeans in Brazil is estimated to be of 24000,000 ha and the production is expected to be around 70,3 millions tones (Conab, 2011). This shift in the production system in Brazil led farmers to further rely on applications of glyphosate, favoring the stiff selection of glyphosate-resistant weed populations.

The first report of resistant biotypes of *C. canadensis* to glyphosate was from the US in 2001 (VanGessel, 2001), and since then multiple reports about this weed resistance have been recorded in numerous countries (Heap, 2011). In Brazil the first resistant population was detected in 2005 in a citrus orchard (Moreira *et al*., 2007; Heap, 2011).

Weed resistance to herbicides can be defined as an inherent capacity and inheritable of certain biotypes, within a population, to survive and reproduce after exposure to doses of herbicide that would be lethal to normal individuals (susceptible) of the same species (Christoffoleti & López-Ovejero, 2008). It is a natural phenomenon inherent to existing populations of certain weeds and, therefore, not induced by the herbicides themselves. Herbicide applications functions rather as a selector of individuals bearing the genes which make them resistant to the chemical which occur in low frequency initially becoming progressively common and may ultimately become the dominant form in the population (Christoffoleti *et al*., 1994).

Numerous studies have been done to understand the resistance mechanism of *C. canadensis* to glyphosate, but only recently the true mechanism involved in this resistance was elucidated, although many of the steps involved are still unknown. Ge *et al*. (2010) proposed that a selective sequestration of glyphosate into the vacuole

confers the observed horseweed resistance. This seems to be the same tactic used in the resistant population of C. *canadensis* which are tolerant to paraquat (Jóri, *et al*., 2002). In the other hand, triazine resistant is due to a mutation in the codon for amino acid 264 of psbA chloroplast gene protein, resulting in the substitution of Serine to Glycine in the photosystem II reaction center of the D1 protein causing the decreased biding of atrazine and Q_b (Szigeti & Lehoczi, 2003; Gawronski et al., 1992; Gressel, 1985).

Reports about interspecific hybridization between *C. canadensis* and *Conyza bonariensis*, another problematic weed in soybean fields, as well as other closely related weeds (Thebaud & About, 1995), suggest that the gene flow may lead to the emergence of hybrids that besides carrying herbicide resistant genes, might have greater fitness than the parental strain, which may means, in the future, increased yield losses and control problems. Although no hybrid with these characteristics have been reported to date, this probability should not be ignored since this is a natural process that promotes genetic diversity and better adaptation of the plant species to the environment. Another cause of concern with regards to *C. canadensis* is the fact that its wind-dispersed seeds are able to move tens or hundreds of kilometers in a single dispersal event, which means that seeds that carried resistant herbicide genes may be dispersing to places where resistant populations have not occurred (Dauer *et al*., 2009). Not only seeds may be involved in carrying resistant genes, but also pollen, thereby connecting farms once thought to be independent (Dauer *et al*., 2007).

When a weed population is selected for herbicide-resistance in a certain area and resistant biotypes lead to failures in chemical control limiting farm production there may be a need for changes in the management practices (López-Ovejero et al., 2006; Moreira *et al*., 2010). One such change might be the adoption of biological control practices, if such alternative exist for the problem weed species.

Biological control of weeds is the deliberate use of natural enemies to reduce the population of a target weed to below a desired threshold (Watson, 1991), and can be divided into two main approaches: classical and bioherbicide. The biocontrol approach using a natural enemy (usually an arthropod or a pathogen) imported from a foreign location (from which the weedy plant is native) to control the plant in the area where it became an invasive weed has been called the classical or the

inoculative biocontrol method. Conversely, the inundative or bioherbicide approach utilizes indigenous plant pathogens that are isolated from weeds and are mass-cultured to produce large numbers of infective propagules. These infective propagules are applied at rates that will cause high levels of infection, leading to suppression of the target weed before economic losses are incurred (Yandoc-Ables *et al*., 2006). When the plant pathogens involved is a fungus, the name used for the product is mycoherbicide (Weaver *et al*., 2007). Annual applications are required since the pathogen does not generally survive in sufficient numbers between growing seasons and not naturally produce the level of inoculum needed to initiate a new epidemic on new weed infestations (Yandoc-Ables *et al*., 2006).

Wilson (1969) noted that "the idea of using plant pathogens to control weeds is almost old as the science of plant pathology itself", but only from the 1970's the plant pathologens started being investigated for use in biological control programs. In a review published twenty years ago, it was acknowledged that although many pathogens had been investigated for weed control none had been commercially successful (Hoagland, 1990). A series of factors have been blamed as reasons for such failures such as technical difficulties related to mass production and formulation, the numerous demands for the commercial products registration and the inadequate choice of target plants (Auld & Morin 1995). Among the logical alternatives suggested for overcoming these problems is the choice of target plants that alone would represent a significant market (Barreto & Evans, 1997), what is the case of *C. canadensis* selected as a target-weed in the present study. In Brazil other plant species such as *Lantana camara* L., *Commelinea benghalensis* L., *Alternantera philoxeroides* (Mart.) Griseb., *Cyperus rotundus* L. and *Euphorbia heterophylla* L., already had been targets of specific programs aimed at developing mycoherbicides for their control and for the last one, a mycoherbicide based in *Lewia chlamidosporiformans* Vieira & Barreto already has its formulation ready and is under registration process (Pereira *et al*., 2003; Lustosa, 2004; Pomella, 2007; Vieira & Barreto, 2010).

Many steps are involved in developing a mycoherbicide, but the first step is always a careful survey of the pathogenic mycobiota associated with the target plant. Although it is not certain whether *C. canadensis* is originally native from Brazil it was known from preliminary observations that a diverse mycobiota exists in

association with that plant species in Brazil allowing for the possible selection of a good mycoherbicide candidate among its components. Thus, the aim of the present work was that of surveying the mycobiota associated *C. canadensis* in Brazil and preliminarily assessing the biocontrol potential of these fungi.

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Mycobiota associated with the weed *Conyza canadensis* in Brazil with particular reference to fungal pathogens for biological control

(According to the guidelines of Mycologia)

Mycobiota associated with the weed *Conyza canadensis* in Brazil with particular reference to fungal pathogens for biological control

Lidiane Leal Duarte

Robert Weingart Barreto

Departamento de Fitopatologia, Universidade Federal de Viçosa, Minas Gerais 36571-000, Brazil

Abstract: A survey of fungi associated with the weed *Conyza canadensis* was conducted in Brazil aiming at finding potential biological control agents. Twelve fungal species were collected, identified, described and illustrated, including: three ascomycetes (*Mycosphaerella* sp., *Sphaerulina* sp. nov. and *Wentiomyces melioloides*), three hyphomycetes (*Alternaria tenuissima*, *Cercospora* sp. nov., and *Cercosporella virgaureae*) one powdery mildew (*Podosphaera fusca*), three coelomycetes (*Colletotrichum capsici*, *Phoma canadensis* and *Septoria erigerontis*), one rust fungus (*Aecidium* sp. nov.) and one oomycete (*Basidiophora entospora*). Three among the fungi that were collected represented new taxa, and the others represented either new host or new geographic records or both, except *Cercosporella virgaureae* that were already recorded on *Conyza canadensis* in Brazil. Preliminary observations suggest that *Phoma canadensis* have the greatest biocontrol potential. It is fast-growing and sporulates well in culture and it is capable of causing plant death or significantly delays plant growth.

Key words: Asteraceae, biological control, fungal survey, taxonomy

Introduction

Conyza canadensis (Asteraceae) is an herbaceous plant native to North America, known mainly by its wide distribution and specially to be a problematic weed of more than 40 crops in 70 countries (Kissmann & Groth, 1999; Holm *et al*., 1997). Due to its numerous features as prolificacy, wind dispersal of seeds, high tolerance to environmental stress and natural resistance of some of its populations to several types of herbicides *C. canandensis* has become a serious large-scale problem in crops, raising its status as a weed and placing it among the world's worst weeds (Weaver, 2001; Heap, 2011). In Brazil the worst problems are presently in soybean and citrus plantations, particulary where glyphosate applications are treated as the sole tool of weed control (Moreira *et al*., 2010; Lamego &Vidal, 2008), which is notably a worrying fact in soybean crops where the so called Roundup-Ready soybeans are used. Since the introduction of these transgenic soybean cultivars, the appearance of resistant populations has deteriorated in Brazil, selecting an increasing

proportion of *C. canadensis* resistant biotypes what resulted in increased failure in chemical control and significant losses.

The soybean *Glycine max* (L.) Merrill is the main crop in Brazil in both volume and income generation, being responsible for 1.5% of total PIB, 11% of domestic exports and for the generation of 1,5 million direct and indirect jobs (ABIOVE, 2010). If in extreme situations of heavy *C. canadensis* infestations the production losses in soybean fields can be of up to 83% (Bruce & Kells, 1990) is urgent a change in management practices, including the possible use of biocontrol methods.

Almost there is no available information about the use of biocontrol agents to control this weed. Charudattan (2001) mentions the bacterial pathogen *Pseudomonas syringae* pv. *tagetis* as having the potential to be used for this purpose, although there is no more further information on the status of this work . Shrestha *et al*. (2008) also mentions that certain stem borers and leaf-eating caterpillars have been observed to damage *C. canadensis*, but again it is not known if those bugs are capable of maintain the weed population levels below the damage. There is no previous research in biocontrol of this weed of world importance with the use of pathogenic fungi. As a starting point for this kind of study aimed at developing a bioherbicide for *C. canadensis* a systematic survey of the pathogenic mycobiota associated with this target plant should be done. Although it is not certain that *C. canadensis* is native from Brazil some ad hoc preliminary observations indicated that a diverse mycobiota exists in association with that plant species in Brazil allowing for the possible selection of a good mycoherbicide candidate among its components. Thus, the aim of the present work was that of surveying and describing the mycobiota associated *C. canadensis* in Brazil, and preliminarily assessing the biocontrol potential of these fungi.

Materials and methods

The samples included in this study were collected during surveys conducted between the years of 2008 and 2010, covering all the southern and southeastern Brazilian states plus the state of Goias in Midwestern Brazil. These were collected in crop and pasture areas as well as in ruderal situations. Survey sites were arbitrarily

selected during the journeys and the areas were searched for the occurrence of populations of the weed. Whenever diseased plants were found representative parts bearing symptoms were taken, photographs were made and notes were taken. Samples were dried in a plant press and later transferred to envelopes. All samples were carefully examined under a dissecting microscope and whenever fungal structures appeared to be associated with disease symptoms (and the fungi were recognized as belonging to culturable taxa) isolations in pure culture were attempted by the directed transfer of spores or other fungal structures onto plates containing VBA - vegetable broth-agar, as described by Pereira *et al*. (2003) with the help of sterile fine pointed needle. Pure cultures were preserved in PCA slants or in silica-gel, as described in Dhingra and Sinclair (1996). Cultures were deposited in the culture collection of the Universidade Federal de Viçosa (COAD) and duplicates were also sent for deposit in the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands). All relevant herbarium samples were deposited in the herbarium of the Universidade Federal de Viçosa (Herbarium VIC).

Culture descriptions were based on the observation of the colonies formed in plates containing potato dextrose agar (PDA) and potato carrot agar (PCA) incubated under 25^{o}C and with a 12 h daily light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates) after 10 days. In the case of *Alternaria* V8-agar plates were also used additionally in the same conditions. For *Phoma canadensis* the culture description were also made by following the protocol described in Boerema *et al*. (2004). Color terminology followed Rayner (1970).

Observations of the fungus morphology were made in slides containing free hand sections or fungal material scraped from the diseased tissues and mounted in lactophenol or lactofucsin. Measurements and illustrations (line drawing and photomicrographs) were prepared with an Olympus BX 50 light microscope fitted with a drawing tube and an Olympus E330 camera. All fungus descriptions are based solely on the fungal structures from field samples, except for *Alternaria tenuissima* and *Phoma canadensis*, for which structures produced in culture were used in descriptions according to the instructions of Simmons (2007) and Boerema *et al*., (2004), respectively.

Patogenicity tests, were performed for demonstrating the pathogenic status of all fungi but the biotrophic fungi (*Aecidium* sp. nov., *Basidiophora entospora*,

Podosphaera fusca, *Wentiomyces melioloides*) plus *Sphaerulina* sp. (for which no culture could be obtained). These consisted of spraying healthy 10 to 15 cm *C. canadensis* plants with a spore suspension, containing at least 2 x 10^5 spores/mL added with Tween 20, until runoff. The exception was Septoria *erigerontis* and *Cercosporella virgaureae* for which culture plugs were used as inoculum because of theirs poor sporulation in culture medium. For *Phoma canadensis* in addition to inoculations involving conidia an attempt was also made of using suspended ground mycelium. Inoculated plants were maintained in a dew chamber for 48 h after inoculation and then transferred to a greenhouse. Re-isolation was performed whenever symptoms were produced and identity of the isolates was checked for confirmation of fulfillment of Koch's postulates.

Additionally, in order to clarify the identity of *Mycosphaerella* and *Cercospora* and to confirm the diagnosis of *Cercosporella virgaureae* and *Septoria erigerontis*, a representative isolate of each was selected and molecular studies were done according to the following steps: the FastDNA kit (BIO 101, Carlsbad, CA) was used according to the manufacturer's instructions to isolate genomic DNA obtained from the fungal mycelia grown on MEA plates for 8 days at 24°C. A sterile needle was used to scrape the mycelia from the surface of the plate. The primers ITS4 and ITS5 (White *et al*., 1990) were used to amplify the ITS areas as well as the 5.8S rRNA gene, of *Cercospora* and *S. erigerontis* isolates. Part of the translation elongation factor (TEF) gene of *Cercospora* and *Mycosphaerella* isolates was amplified using the primers EF728F and EF-2 (Carbone & Kohn, 1999), and a part of the calmodulin (CAL) gene were amplify using the primers CAL228F and CAL737R (Carbone & Kohn, 1999) for *Cercospora* isolate only. The primers LSU forward and LR5 were use to amplified parte of the large subunit ribosomal for *C. virgaureae* and *Mycosphaerella*. The polymerase chain reaction conditions were the same for all regions and the reaction mixture had a total volume of 12.5 µl: 1 µl of diluted gDNA, 1× PCR buffer, 48 µM each of the dNTPs, 2.5 pmol of each primer, 1.5 mM of $MgCl_2$ and 0.7 units Taq polymerase (Bioline GmbH, Luckenwalde, Germany). The amplification reactions were done on a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT). The initial denaturation step was done at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C (30 s), annealing at 52°C (30 s), and elongation at 72°C (30 s). A final elongation step at 72°C (7 min) was included

in the run. The PCR products were separated by electrophoresis at 80 V for 40 min on a 0.8% (wt/vol) agarose gel containing ethidium bromide at 0.1 µg/ml in 1× Tris-acetateEDTA buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualized under UV light.

The amplicons were sequenced in both directions using the PCR primers and a DYEnamic ET Terminator Cycle Sequencing kit (Amersham Biosciences, Roosendal, The Netherlands) according to the manufacturer's recommendations. The products were analyzed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Foster City, CA). A consensus sequence was computed from the forward and reverse sequences with SeqMan from the Lasergene package (DNAstar, Madison, WI).

The sequences of *Mycosphaerella* and *S. erigerontis* were only compared with the available sequences in GenBank whereas the sequence of *Cercospora* and *C. virgaureae* were used in phylogenetic studies.

For these, the sequences were assembled and added to the outgroups using MEGA 5.0 software package and were manual adjusted for improvement by eye where necessary. Maximum Likelihood and Bayesian analyses were performed for all data (Tables 1 and 2) and consisted of neighbor-joining analysis with the Jukes-Cantor substitution model. Alignment gaps were treated as missing data and any ties were broken randomly when encountered. The best-fit evolutionary model was determined for each data set by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10; Sinauer Associates, Sunderland, MA) and MrModeltest 2.2 (Nylander, 2004) for bayesian analyses or Modeltest 3.7 (Posada & Grandall, 1998) for Maximum Likelihood (ML) analyses. The robustness of trees in the ML was evaluated by 1000 bootstrap replications. The Bayesian analysis was performed using Markov chain Monte Carlo sampling and were run with two chains 1,000,000 generations by which time the average standard deviation of split frequencies was close to or less than 0.01. The program Tracer v1.5 (Rambaut & Drummond, 2003) was used to ensure the convergence of the chains and then the consensus tree was calculated in MrBayes v3.1 (Huelsenbeck & Ronquist , 2001) where the first 25% of sampled trees were discarded as ''burnin''. Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut, 2009).

Results

Conyza canadensis was observed to be particularly serious in southern Brazil where infested fields were commonly found. Interestingly it was also in southern Brazil that the more diverse mycobiota was collected on this plant. A total of twelve different species of fungi considered as likely or obvious pathogens of *C. canadensis* was collected and are described below.

Aecidium sp. nov. Fig. 1

Lesions on living leaves, observed initially as yellow-green depressions on adaxial leaf surface and small, smooth swellings on the lower surface of the leaves, that later developed into yellow to orange pustules formed abaxially with 4-10 mm diam. External mycelium absent. Internal mycelium intercellular, up to 3.0 µm diam, branched, septate, cylindrical and hyaline. Spermogonia amphigenous, subepidermal, type V, appearing as a group of black dots surrounded by aecia. Aecia in chlorotic, circular lesions forming concentric circles around spermogonia, hypophyllous, erumpent, cylindrical with the edges curved and lacerated, 135.0–218.5 µm diam. Peridium wall developed, membrane up to 17.5 µm thick, cells firmly attached in regular rows, polyhedral, sub rhomboid to sub rectangular in shape, outer surface verrucose, inner surface striate, 10.5–24.5 × 11.5– 25.5 µm, ocher to sub hyaline when fresh and light brown in older samples. Aeciospores catenulate, subglobose to slightly ellipsoid, 14.0–24.5 × 13.0–19.5 µm, aseptate, walls 1.0–1.5 µm thick laterally and 1.0- 4.0 µm thick apically, content orange, minutely verrucose, wall hyaline. Uredinia and telia unknown.

Material examined: Brazil, state of São Paulo, São Paulo, on living leaves of *Conyza canadensis*, 24 April 2010, E. Guatimosim (VIC 31617). Brazil, state of Paraná, Nova Prata do Iguaçu, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31646). Brazil, state of Santa Catarina, Cocal do Sul, on living leaves of *Conyza canadensis*, 17 October 2010, E. Guatimosim (VIC 31645). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634). Brazil, state of Santa Catarina, Iaguará do Sul, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31636). Brazil, state of Goias, Rio Verde, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31640).

Notes: The Pucciniales (formerly Uredinales) includes more than 7.000 species found on a wide range of wild and cultivated plants in many parts of the world (Kolmer, 2009) and are obligate parasites having some of the most complex life cycles amongst fungi (Hiratsuka & Sato, 1982) many of which are uncompletely known or have parts of their cycle described separately having their connections unresolved. In Brazil, Hennen *et al* (2005) recognized more than 800 species, belonging to 56 holomorphic and 9 anamorphic genera.

Several rusts have been described in association with members of *Conyza*, (Farr *et al*., 2011; Hennen *et al*., 2005), although only a few have the aecial phase. The fungus on *C. canadensis* collected during this survey differs from *Aecidium conyzae-colombiensis* Pardo-Cardona, *A. conyzae-pinnatilobatae* P. Syd. & Syd., *A. hoffmannii* P. Syd. & Syd. and *A. niederleinii* Henn.by its shorter peridium cells and also differs from A. *conyzae-pinnatilobatae* by its much smaller aecia and A. *niederleinii* by its peridium cells shape. For more details see table 3. *Puccinia alia* H.S. Jacks. & Holw. differs from *Aecidium* sp. nov. by its lacking a peridium while *P. cyperi* Arthur differs by its larger spores and *P. dioicae* Magnus by its indistinct spermogonia. Among the anamorphic genera of rusts *Aecidium* is one of the easiest to recognize. Nevertheless, separation of different species of *Aecidium* is often challenging because of their coincident morphological features (Hennen *et al*., 2005). However, it was regarded here that in the present case morphology provided enough information to justify the proposal of a new name for the fungus on *C. canadensis* from Brazil.

Although common, *Aecidium* sp. was not found in some of the states covered during the survey. It causes cup-shaped foliar deformations and yellowing of infected tissues that later led to necrosis. However, lesions are usually not abundant and impact appears to be minimal indicating that it has no significant biocontrol potential.

Alternaria tenuissima (Nees) Wiltshire*, Transactions of the British Mycological Society* 18(2): 157 (1933) Fig. 2

Lesions on living leaves starting as small necrotic spots becoming irregular with age and eventually coalescing and leading to necrosis of large portions of

leaves. External mycelium absent. Internal mycelium indistinct. Stromata absent. Conidiophores epigenous, solitary, cylindrical, straight to slightly sinuous, 39.0–119.0 × 4.0–5.0 μm, 2–10 septate, light brown, smooth. Conidiogenous cells terminal, integrated, proliferating sympodially, cylindrical. Conidiogenous loci conspicuous, darkened, thickened. Conidia catenulate (conidial chains long and sparingly branched), obclavate to ovoid (obclavate conidia with 4–6 transverse septa and 1–2 longitudinal septa, 38.5–64.0 × 11.0–16.5 μm; ovoid conidia with 0–3 transverse septa and 1–7 longitudinal septa, 30.0–62.0 × 11.0–18.0 μm), golden brown, slightly verruculose.

In culture: slow growing (5.5 cm diam in 10 days on PDA and V8, average but faster-growing in PCA - 6.5 cm diam in 10 days), colony flat, aerial mycelium felty, sporulating, strong diurnal zonation, alternatingly smoke grey and mouse grey haloes in PCA, pale olivaceos and grey olivaceous in V8, smoke grey and grey olivaceous on PDA; halos of smoke grey and pale olivaceous grey reverse on PCA and V8, on PDA olivaceous and grey olivaceous.

Material examined: Brazil, state of São Paulo, Mairiporã, on living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31618).

Notes: *Alternaria tenuissima* is morphologically very close to *A. alternata* (which has already been reported on *C. canadensis* as the teleomoph *Clathrospora diplospora* (Ellis & Everh.) Wehm. (Farr *et al*., 2011)), but differ from the latter by its longer conidial chains and darker conidia with shorter beaks. The association between *A. tenuissima* and *C. canadensis* has already been reported from China but this is the first record of this association in Brazil.

There are several *Alternaria* species used or with potential to be biocontrol agents as *A. cassia* Jurair & A. Khan and *A. eichhorniae* Nag Raj & Ponnappa (Ávila et al., 2000; Shabana *et al*., 1997), and as our isolate readily sporulates in culture medium, as well was capable of causing necrosis, leaf distortion and considerable delay the development of its host in the in pathogenicity tests, initially we consider it as a possible biocontrol agent, but ironically, despite the promising results obtained, this species is a known pathogen of both citrus plants and soybean (Jasnic *et al*., 2011; Farr *et al*., 2011) and unless this isolate proves to be specific to *C. canadensis* (which appears unlikely) a mycoherbicide based on it would not be appropriate for two of the high potential markets: citrus and soybean plantations.

Basidiophora entospora Roze & Cornu, *Annales des Sciences Naturalles*, Paris Séries 5, 11:89 (1869) Fig. 3

Lesions on living leaves, infected tissues initially bearing a diffuse chlorosis becoming yellowish adaxially, bearing abundant downy whitish sporulation abaxially, becoming necrotic and leading to extensive blight with age. External mycelium absent. Internal mycelium indistinct. Sporangiophores hipophyllous, emerging from the stomata, clavate-muricate, straight, unbranched, cylindrical, ending in swollen head bearing 6–13 sterigmata, 104.0–268.0 × 10.5–18.0 (diam at trunk), diam at head 16.0–32.0 µm, sterigmata 5.5–11.0 × 2.5- 3.5 µm, hyaline, smooth. Sporangia globose to ellipsoid, 24.5–37.5 × 19.5–36.5 µm, solitary on each sterigmata, hyaline, smooth. Oospores not observed.

Material examined: Brazil, state of Rio Grande do Sul, Marau - Casca, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31650). Brazil, state of Rio Grande do Sul, Marau, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31611). Brazil, state of Paraná, Concordia, on living leaves of *Conyza canadensis*, 15 october 2010, E. Guatimosim (VIC 31609). Brazil, state of Rio Grande do Sul, Erechim, living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31653). Brazil, state of Rio Grande do Sul, Erechim – Passo Fundo, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31626). Brazil, state of Rio Grande do Sul, Bento Gonçalves, on living leaves of *Conyza canadensis*, 16 October 2010, E. Guatimosim (VIC 31652). Brazil, state of Rio Grande do Sul, Iaquara, on living leaves of *Conyza canadensis*, 16 October 2010, E. Guatimosim (VIC 31621). Brazil, state of Rio Grande do Sul, Gramado, on living leaves of *Conyza canadensis*, 16 October 2010, E. Guatimosim (VIC 31651). Brazil, state of Santa Catarina, Cocal do Sul, on living leaves of *Conyza canadensis*, 17 October 2010, E. Guatimosim (VIC 31654). Brazil, state of Santa Catarina, Anitápolis, on living leaves of *Conyza canadensis*, 17 October 2010, E. Guatimosim (VIC 31630). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31627). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634). Brazil, state of Santa Catarina, Iaguará do Sul, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31636). Brazil, state of São Paulo, Iuquiá, on living leaves of *Conyza canadensis*, 19 October 2010, E. Guatimosim (VIC 31631).

Notes: The genus *Basidiophora* was first described by Roze and Cornu in 1869 and has so far only two species: *B. entospora* Roze & Cornu and *B. montana* R.W. Barreto, that are characterized by being obligate parasites and by its host range

largely restricted to plants of the Asteraceae (Cunnington & Constantinescu, 2006). Our biometric data indicated that our isolates fits well within the *Basidiophora entospora* and that it has a restrict distribution within the sampling sites, being present only in the southern states plus São Paulo and Minas Gerais. *Basidiophora entospora* has already been recorded in *C. canadensis* in various parts of the world (Farr *et al*., 2011); nevertheless this is the first report of these fungi in leaves of *C. canadensis* in Brazil.

Although specific to Asteraceae and causing a disease which can be rather severe it is clearly inadequate for biological control since on one side it is a biotrophic of no interest for mycoherbicide development and on the other side it is inadequate as a classical biocontrol agent as it already occurs in many parts of the world without clearly contributing to mitigating invasions of crop areas.

Cercospora sp. nov. Fig. 4

Lesion on living leaves, distributed randomly over the leaf, starting as necrotic dark brown dots and becoming sub-circular, partly angular to irregular spots, larger lesions (up to 8.7 mm diam) dark brown with a grayish centre and, at times, with a diffuse chlorotic peryphery, External mycelium absent. Internal mycelium intercellular, 3.0–4.0 µm diam, sparingly branched, subcylindrical, septate, light brown to subhyaline. Stromata substomatal, either reduced to a few cells or well developed, oblate to spheroidal, 10.5–41.0 µm diam, composed of textura angularis, medium brown. Conidiophores hypogenous, arising through stomata in loose, sparse fascicles (maximum of ten conidiophores), cylindrical, 0-3 geniculations, straight becoming sinuose towards the apex, (56.5–) 64.0–115.5 (–155) × 4.5–8.0 µm, 0–5 septate, dark brown, smooth. Conidiogenous cells holoblastic, terminal or intercalary, integrated, prolifering sympodially, cylindrical. Conidiogenous loci conspicuous, thickened and pigmented. Conidia solitary, obclavate to filiform, straight to slightly curved, 2.5–5.5 × 35.5–237.0 µm, apex subacute, base truncate, 2.5–4.0 µm, 1–14 septate, hylum thickened and darkened, gutulate, hyaline, smooth.

In culture: On PDA and PCA slow-growing (3 cm diam in 10 days); colony flat, aerial mycelium abundant in PDA, scarce centrally and denser cottony to powdery towards the periphery in PCA, glaucous in PDA, pale greenish gray in

PCA, slightly raised centrally, moist, suffusing the medium with vinaceous pigment; lived red reverse on PDA and dark vinacea on PCA; no sporulation.

Phylogeny: Sequences obtained for the selected isolate from *C. canadensis* were aligned with sequences of *Cercospora* retrieved from the GenBank as well as to three outgroup sequences. The alignment data matrix consisted: for the ITS region, of 54 taxa and 439 characters, in which 35 sites were variable and only 32 sites were parsimony informative; for the CAL gene, 59 taxa were used, of 265 characters, 85 were variable which 78 were parsimony informative; for to the TEF gene, 55 taxa were used, of 252 characters, 83 were variable which 80 were parsimony informative.

Material examined: Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 14 January 2010, L. L. Duarte, (VIC 31269). Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 20 January 2010, L. L. Duarte, (VIC 31670). Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 27 January 2010, L. L. Duarte, (VIC 31671). Brazil, state of Minas Gerais, Pouso Alegre - Poços de Caldas, on living leaves of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31681). Brazil, state of Paraná, Capivari, on living leaves of *Conyza canadensis*, 12 october 2010, E. Guatimosim (VIC 31657). Brazil, state of Paraná, Mamborê, on living leaves of *Conyza canadensis*, 13 october 2010, E. Guatimosim (VIC 31682). Brazil, state of Paraná, Corbelia - Cascavel, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31612). Brazil, state of Paraná, Nova Prata do Iguaçu, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31683). Brazil, state of Paraná, Maringá, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31614). Brazil, state of Paraná, Janiópolis, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31608). Brazil, state of Paraná, Capivari – Vale do Sonho, on living leaves of *Conyza canadensis*, 17 october 2010, E. Guatimosim (VIC 31622). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634). Brazil, state of São Paulo, Amparo, on living leaves of *Conyza canadensis*, 19 October 2010, E. Guatimosim (VIC 31610). Brazil, state of Minas Gerais, Unaí – Cristalina, on living leaves of *Conyza canadensis*, 28 November 2010, L. L. Duarte, (VIC 31675). Brazil, state of Goias, Aparecida, on living leaves of *Conyza canadensis*, 03 December 2010, L. L. Duarte, (VIC 31678). Brazil, state of Goias, Gouvelãndia - Inaciolãndia, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31676). Brazil, state of Goias, Cachoeira Dorada, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31677). Brazil, state of Goias, Santa Helena - Quirinópolis, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31637). Brazil, state of Goias, Jatai - Rio Verde, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31672). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31680). Brazil, state of Minas Gerais, Ibiá, on

living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31639). Brazil, state of Minas Gerais, Patrocinio - Ibiá, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31673). Brazil, state of Minas Gerais, Ibiá - São Gotardo, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31674). Brazil, state of Minas Gerais, Patrocinio, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31679).

Notes: All the morphological characteristics evaluated for all the *Cercospora* isolates from *C. canadensis* were equivalent to those described for *C. apii* sensu lato in Crous and Braun (2003), which initially seemed to indicate that these isolates should be placed within *Cercospora apii*. Nevertheless, the phylogenetic analysis has clearly shown that this would be inadequate. Although data for the ITS alone (Fig 5, 6) would favour such placement, analysis of CAL (Fig 7, 8) and TEF (Fig 9, 10) has shown that the isolate from *C. canadensis* does not group within any the *C. apii* Fresen. emend. Crous & U. Braun *sensu lato* species. Although the trees topologies generated for CAL and TEF genes, for both Bayesian and Maximum Likehood analysis, were not equivalent both confirmed, the placement of *Cercospora* sp. nov. as a distinct species within the *C. apii* complex (with greater support from CAL gene).

Our results are in agreement with and support the results of a previous work (Groenewald *et al*., 2005) which rejected the hypothesis that all morphologically indistinguishable *Cercospora* forms belonging to the *C. apii*-complex represented a single species. As it is impossible to distinguish morphologically the fungus on *C. canadensis* from similar species of *Cercospora*, molecular and host information are then necessary for recognition of the newly proposed species.

This fungus was recognized as having a wide distribution in Brazil, occurring in all states covered during the survey. Its pathogenicity was demonstrated and extensive necrosis of inoculated leaves resulted but infections did not kill the apical meristem and infected plants recoved later. Conidial production for this fungus, as is commonly the case for cercosporoid fungi, was scarce and it appears, at this stage, that this fungus is not a good candidate for use as a mycoherbicide.

Cercosporella virgaureae (Thüm.) Allesch, *Hedwigia* 34:286 (1895), Fig. 11

Lesions on living leaves, starting as necrotic and circular spots and later becoming elongated along the main leaf axis, sub-angular to irregular, 0.2 a 7.5 mm diam. External mycelium absent. Internal mycelium intercellular, 3.0–4.0 µm diam, branched, cylindrical, septate, subhyaline. Stromata well developed, substomatal, globose to subglobose, 36.0–70.0 × 43.5–64.0 µm diam, composed by sub hyaline and textura angularis cells. Conidiophores hypogenous, in sparse fascicles, arising through stomata, cylindrical, straight or occasionally curved, 22.0–64.0 × 4.0–6.5 µm, unbranched, 0–1 septate, sub hyaline, smooth. Conidiogenous cells holoblastic, terminal, integrated, proliferating sympodially, cylindrical, 32.0–63.0 × 4.0–5.0 µm, subhyaline. Conidiogenous loci conspicuous, thickened and non-pigmented. Conidia obclavate-cylindric, straight to very slightly curved or sinuose, 28.0–75.0 × 5.0–5.5 µm, broadly rounded at the ends, thin-walled, 1–7 septate, gutulate, sub-hyaline, smooth.

In culture: slow-growing (0.6-1.5 cm diam in 2 months); colonies stromatic with sparse aerial mycelium over raised irregular center and some cottony to powdery mycelium at periphery, white, moist, pigmenting the medium around the colony; no sporulation. Reverse: On PCA center black surrounded by a grayish sepia halo and on PDA dark mouse grey, very homogenous.

Phylogeny: The alignment of isolate *Cercosporella* BSV 09 LSU region sequence with other 45 Mycospharellaceae sequences retrieved from GenBank plus the outgroup resulted in the follow data matrix: 888 characters, in which 141 were sites variable and these 72 were parsimony informative.

Material examined: Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 07 January 2010, L. L. Duarte, (VIC 31641).Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 20 January 2010, L. L. Duarte, (VIC 31718). Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 27 January 2010, L. L. Duarte, (VIC 31721). Brazil, state of Minas Gerais, Caldas, on living leaves of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31607). Brazil, state of Minas Gerais, São João Del Rey - Tiradentes, on living leaves of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31620). Brazil, state of São Paulo, Água da Prata, on living leaves of *Conyza canadensis*, 22 April 2010, E. Guatimosim (VIC 31615) Brazil, state of São Paulo, Limeira, on living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31736). Brazil, state of São Paulo, Mairiporã, on living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31738). Brazil, state of São Paulo, Mairiporã, on

living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31618). Brazil, state of São Paulo, Mairiporã, on living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31618). Brazil, state of Minas Gerais, São Geraldo, on living leaves of *Conyza canadensis*, 23 June 2010, L. L. Duarte, (VIC 31722). Brazil, state of Minas Gerais, São Geraldo, on living leaves of *Conyza canadensis*, 23 June 2010, L. L. Duarte, (VIC 31724). Brazil, state of Minas Gerais, Visconde do Rio Branco, on living leaves of *Conyza canadensis*, 26 June 2010, E. Guatimosim (VIC 31737). Brazil, state of Paraná, Ponta Grossa - Guarapuava, on living leaves of *Conyza canadensis*, 12 october 2010, E. Guatimosim (VIC 31628). Brazil, state of Paraná, Nova Prata do Iguaçu, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31733). Brazil, state of Rio Grande do Sul, Vila Maria, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC). Brazil, state of Rio Grande do Sul, Erechim, living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31735). Brazil, state of Rio Grande do Sul, Iaquara, on living leaves of *Conyza canadensis*, 16 October 2010, E. Guatimosim (VIC 31621). Brazil, state of Santa Catarina, Cocal do Sul, on living leaves of *Conyza canadensis*, 17 October 2010, E. Guatimosim (VIC 31734). Brazil, state of Santa Catarina, Anitápolis, on living leaves of *Conyza canadensis*, 17 October 2010, E. Guatimosim (VIC 31630). Brazil, state of Paraná, Capivari – Vale do Sonho, on living leaves of *Conyza canadensis*, 17 october 2010, E. Guatimosim (VIC 31622). Brazil, state of Santa Catarina, Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31635). Brazil, state of Santa Catarina, Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634). Brazil, state of Santa Catarina, Pomerode, on living leaves of *Conyza canadensis,* 18 October 2010, E. Guatimosim (VIC 31732). Brazil, state of Santa Catarina, Iaguará do Sul, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31636). Brazil, state of Santa Catarina, Guaramirim, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31731). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634). Brazil, state of São Paulo, Itatiba, on living leaves of *Conyza canadensis*, 19 October 2010, E. Guatimosim (VIC 31730). Brazil, state of São Paulo, Iuquiá, on living leaves of *Conyza canadensis*, 19 October 2010, E. Guatimosim (VIC 31631). Brazil, state of Goias, Aparecida, on living leaves of *Conyza canadensis*, 03 December 2010, L. L. Duarte, (VIC 31727). Brazil, state of Goias, Inaciolândia - Itubiara, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31726). Brazil, state of Goias, Pantaninho on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31606). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31717). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31716). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31705). Brazil, state of Minas Gerais, Ibiá, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31639). Brazil, state of Minas Gerais, São Gotardo, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31720). Brazil, state of Minas Gerais, Patrocinio - Ibiá, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC

31719). Brazil, state of Minas Gerais, Patrocinio, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31728).

Notes: *Cercosporella virgaureae* is the type species for the genus *Cercosporella.* The name *Ramularia virgaureae* Thümen was proposed in 1874 for a fungus on *Solidaginis virgaureae* L. collected in Austria. Independently, the genus *Cercosporella* was erected by Saccardo (1880) for a single species, *Cercosporella cana* in *Erigeron canadensis* (today *Conyza canadensis*), which was later found to be conspecific with the earlier *R. vigaureae* and then recombined as *C. vigaureae* by Allescher (1895). For its morphological features, the *Cercosporella* collected on *C. canadensis* fits well within *Cercoporella virgaureae*, what was strongly supported by the molecular data (Fig 12, 13). The tree topologies obtained with Bayesian analysis were similar to those based on Maximum Likehood and our isolate grouped with *C. virgaureae* in a clade highly support. The *C. virgaureae* clade was closer to the one of *Cercospora apii* sensu lato than to the *Ramularia* clade, what was surprisingly due to the difficulty of separating these two genera based solely in the morphological characters. *Cercosporella virgaureae* seens to be a cosmopolitan species occurring in most places where *C. canadensis* has been recorded, including Brazil where was first reported by Braun & Freire in 2004.

Cercosporella virgaureae is widely distributed in Brazil occurring in every state included in this study. The pathogenicity test resulted in necrosis and abundant sporulation of this fungus on the lower surface of the leaf where the mycelium plugs were applied. Although other species of *Cercosporella* (namely *C. acroptili* (Bremer) U. Braun and *C. centaureicola* Berner, U. Braun & Eskandari) have already been regarded as having potential for use in weed biocontrol (Berner *et al*., 2005) this is clearly not the case for *C. virgaureae.* This fungus seems to be ubiquitous wherever *C. canadensis* occurs and is hence of no use for classical biological control. Additionally, although field damage appears significant, natural epiphytotics are clearly not sufficient to restrict the impact of weed infestations and possibilities of increasing it through mass mycoherbicide applications doesn't appear feasible as growth in culture is extremely slow.

Colletotrichum capsici (Syd. & P. Syd.) E.J. Butler & Bisby, *Fungi of India*: 152 (1931); Fig. 14

Lesion on living leaves, amphigenous, starting as small necrotic spots which later evolve and coalesce resulting in necrosis irregularly shaped, 0.5–5.0 mm. External mycelium absent. Internal mycelium indistinct. Conidiomata acervular, amphigenous, subcuticular, 44.5–79.5 µm diam. Setae abundant, mostly uniformly medium to dark brown but lighter apically when larger, cylindrical, slightly swollen at base, tapering apically to subacute apex, straight to slightly curved, 1–3 septate, 60.5–158.5 × 3.5–5.0 µm. Conidiophores restricted to the conidiogenous cells, cylindrical, phialidic, (–11.0) 15.0–22.0 × 3.5–7.0 µm, aseptate, unbranched, hyaline, smooth. Conidia falcate with acute ends, 21.0–31.5 × 4.0–5.0 µm, aseptate, gutulate, hyaline to sub hyaline, smooth. Apressoria (observed in slide cultures) borne on hyaline thin-walled supporting hyphae, long clavate to irregular lobate, solitary 12.5–23 × 8.0–13.5 µm, medium brown.

In culture: fast-growing (7.5 cm diam in 10 days), colony flat of well developed aerial mycelium, setae abundant; on PDA powdery, pale olivaceous grey, on PCA cottony, moist, smoke grey, white periphery; sporulation abundant. Reverse: on PDA greenish black alternate with iron grey; on PCA smoke grey only.

Material examined: Brazil, state of Goias, Rio Verde, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31640).

Notes: *Colletotrichum capsici* was originally described as *Vermicularia capsici* by Sydow in 1913 and it has been reported from at least 176 host-genera since then (Roberts & Snow, 1990). Its diagnostic morphological features are large, hyaline and falcate conidia produced on conidiophores asepate, cylindrical which are restricted to phialidic conidiogenous cells, and by its abundant setae (Mordue, 1971; Sutton, 1992). The fungus on *C. canadensis* clearly fits the morphological delimitation of *C. capsici*. There are numerous recors of *C. capsici* on members of the Asteraceae but this is its first record on a *Conyza* (Farr *et al*., 2011). Although *C. capsici* is a well known pathogen in many crops (eg.: cotton, cowpea, pepper, tomatoes and several tropical fruits) (Pring *et al*., 1995), the attempts made to demonstrate the pathogenicity of our isolate to *C. canadensis* failed, suggesting that this is only a secondary pathogen or saprophyte on this plant species and, therefore, of no interest for biological control.

Mycosphaerella sp. Fig. 15

Lesion on living leaves, starting as chlorosis that later developed into necrosis in the oldest parts, where is possible to observe a few fruiting bodies adaxially. External mycelium absent. Internal mycelium intercellular, 5.0–6.0 µm diam, cylindrical to subcylindrical, sparingly branched, hyaline to subhyaline. Ascomata pseudotecial, hypophyllous, subepidermal, solitary, globose to subglobose, 99.0–104.0 × 108.5–123.5 µm, walls of textura angularis, 2-cell thick, medium brown and darker near the ostiole. Deiscence ostiolar, central, one per ascoma, circular, 20.0–27.0 diam. Lack of interthecial filament. Asci bitunicate, parallel, clavate to cylindrical, 37.5–52.5 × 10–14 µm, 8-spored. Ascospores biseriate to inordinate, fusoid-ellipsoidal, 12.5–16.5 × 3.0–5.0 µm, 1-septate, slightly constricted at septum septum median or sub-median making the upper cell slightly larger than the lower, guttulate, hyaline, smooth.

In culture: relatively fast-growing (8 cm diam in 10 days), colony flat, aerial mycelium sparse, cottony and moist, olivaceous brown on PCA and buff on PDA; iron grey reverse on PCA and medium brown on PDA; no sporulation.

Sequences: Comparison of sequences of both translation elongation factor (TEF) and large subunit ribosomal (LSU) with the available sequences in GenBank showed a closest match with *Phoma* species. For TEF, the sequence with a higher similarity were *Phoma medicaginis* Malbr. & Roum. (GenBank accession number HM157757) with 94% of nucleotide homology (over 82% of query coverage) and for LSU, our isolate shares 99% of identity, over a 100% of query coverage, with *Phoma herbarum* Westend. (GenBank accession number AY293791).

Material examined: Brazil, state of Rio Grande do Sul, Erechim – Passo Fundo, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31626). Brazil, state of Rio Grande do Sul, Vila Maria, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31648).

Notes: *Mycosphaerella* is one of the largest genera of Pezizomycetes, comprising more than 1.000 species that have adapted to niches ranging from plant pathogens, saprobes, hyperparasites and even species which are pathogenic to humans (Crous, 2007). The fungus on *C. canadensis* has a morphology which is typical of members of *Mycosphaerella* sp., however our molecular data suggest that *Didymella* (Anamorph: *Phoma*) might actually be the correct generic placement for

the fungus on *C. canadensis*. Observation of the anamorph would help to clarify the identity of this fungus, but unfortunately, in this particular case, no direct connection with possible anamorphs was found during the survey (namely: *Cercospora, Cercosporella, Phoma, Septoria*). Also no anamorph was produced in culture. Nevertheless, Blast-searches in GenBank only give an approximate ideia of what the sequences might be close to and do not allow for a final decision about the placement of a taxon. As morphology indicated that the fungus is not a *Didymella* (since it not has pseudoparaphysis) we decided that, for the moment to place the fungus on *C. canadensis* in *Mycosphaerella*.

There are numerous species of *Mycosphaerella* recorded in association with the Asteraceae (Farr *et al*., 2011) and the limited morphological features available for species separation combined with the common overlapping of morphological features among separate species renders their recognition at the species level very challenging. For these reason an accurate phylogenetical study should be performed to ascertain the identity of this fungus.

Mycosphaerella sp. was found only twice during this survey and only in the state of Rio Gande do Sul, southern of Brazil. Symptom observed in fresh leaves were weak and apparently the fungus do not cause any significant damage to infected plants. Inoculations performed under controlled conditions did not result in any symptom development. Such a failure may have been caused by the use of mycelium as inoculum (which might be not infective), the fungus having lost pathogenicity in culture, incompatible host biotype/fungus strain association among others. At this stage *Mycosphaerella* sp. appears inadequate for use in biological control.

Phoma canadensis Allesch, *Berichte der Bayerischen Botanischen Gesellschaft* 4: 32 (1896); Fig. 16

External mycelium absent. Internal mycelium indistinct. Conidioma pycnidial, amphigenous, subcuticular, solitary, scattered in necrotic tissues, sub globose, walls of textura angularis, 60.0–80.0 × 67.5–95.0 µm, 1–2 cells thick, 7.0–10.0 µm, medium brown. Dehiscence not observed. Conidiophores reduced to the conidiogenous cell, enteroblastic, lageniform or ampulliform, 9.0–13.0 × 6.0–12.0 µm, unbranched, straight, hyaline, smooth. Conidia ellipsoid to sub cylindrical,

straight to slightly curved, 4.0–5.0 × 2.0 µm, aseptate, ends rounded, with 2 small polar guttules, subhyaline, smooth.

In vitro description:

Pycnidia superficial on the culture medium, spherical and sometimes distortedly sub spherical, 82.5–182.5 × 67.5–130.0 µm, walls of textura angularis, honey when immature becoming medium brown. Deiscence ostiolar, central, one per conidioma. Conidia ovoid to cylindrical, straight to slightly curved, 3.0–5.0 × 1.5–2.0 µm, ends rounded, sub hyaline, aseptate, smooth, with 2 small polar guttules.

In culture: slow-growing on OA and MEA (average growth 8 cm diam in 14 days), aerial mycelium sparse, cottony in the middle and felty at the edges, moist and uniformly dark honey; on MEA, aerial mycelium velvety and felty at the periphery, smoke grey centrally, followed by a pale mouse grey ring and finally a grey olivaceousperyphery;on PDA fast-growing (6 cm in 7 days), colonies flat, composed of sparse aerial mycelium cottony in the middle and felty at the edges grey olivaceous centrally and mouse grey at peryphery; colonies on PCA similar to PDA except for the powdery texture; sporulation only observed on MEA. Reverse: on OA Isabeline centrally and Hazel at the periphery; on MEA center dark brown surrounded by an hazel edge; on both PDA and PCA bands of leaden black interspersed with leaden grey.

Material examined: Brazil, state of Minas Gerais, Caldas, on living stems of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31607).

Notes: *Phoma* is a cosmopolitan genus characterized by the production of single celled, hyaline conidia in monophialidic, doliiform to flask-shaped conidiogenous cells in thin-walled pycnidia (Irinyi *et al.*, 2007; Aveskamp *et al.*, 2009). Many species have been reported from a wide range of substrates and plant hosts where they reside as primary pathogens, opportunist, saprobes or endophytes (Aveskamp *et al.*, 2009). The sole species of *Phoma* recorded on *Conyza canadensis* is *P. canadensis* (Farr *et al.*, 2011). This is an obscure species described in the 19th century from Bavaria (Germany) in *Erigeron canadensis* and the only other record of this species is one by French (1989). It is even absent from the world monograph on the genus: *Phoma* identification manual by Boerema *et al.*, (2004) and recent studies on the genus by Gruyter *et al.*, (2009) and Aveskamp *et al.*, (2009). When compared

with the original description the Brazilian isolate differs from it in conidial size (conidia 2 µm diam as compared with 0.5-1 µm in the original description). But size provided in the original description appears to be unrealistically small and considering other features and the common host we preferred to use the existing name for the Brazilian isolate. This is the first record of this fungus in Brazil.

This fungus was found only once during this study associated with small stem lesions and nearly escaped observation. In pathogenicity tests the plants of *C. canadensis* that the grounded mycelium were sprayed on, showed, right after being removed from the humid chamber, leaf necrosis and wilting. Seven days after the inoculation some plants were already dead and the others exhibited extensive necrosis in both leaf and stem and under the necrotic tissue were possible to observe small structures resembling black dots that when examined under a magnifying glass proved to be the pycnidia. One month after the inoculation the plants that remained alive had recovered from infection but had only a third of the height of control plants. Inoculations with a spore suspension not resulted in any symptom development.

Phoma canadensis proved to be able to cause severe damages like significant delay in the development or even death of its hosts when its grounded mycelium is applied, so there are good chances that this might be a potential agent for a mycoherbicide development.

Podosphaera fusca (Fries) U. Braun & Shishkoff, *Schlechtendalia* 4: 29 (2000); Fig. 17

Colonies powdery mainly epyphylous, associated to chlorosis and yellowing of infected leaves. External mycelium loose, 6.0-10.0 µm diam, branched, septate, hyaline, bearing slightly nipple-shaped appressoria. Conidiophores cylindrical, erect, straight, 70.5–110.0 × 11.0–13.0 µm, hyaline. Conidia in short to long chains (2–7), doliiform to ovoid, 27.0 –46.0 × 14.0–19.0 µm (ratio l/w 1.5–2.4), hyaline, smooth, with fibrosin bodies.

Material examined: Brazil, state of Minas Gerais, São João Del Rey - Tiradentes, on living leaves of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31620). Brazil, state of São Paulo, Aguas da Prata, on living leaves of *Conyza canadensis*, 22 April 2010, E. Guatimosim (VIC 31615). Brazil, state of São Paulo, Mairiporã, on living leaves of *Conyza canadensis*, 23 April 2010, E.

Guatimosim (VIC 31685). Brazil, state of Minas Gerais, Viçosa, on living leaves of *Conyza canadensis*, 23 June 2010, L. L. Duarte, (VIC 31692). Brazil, state of Paraná, Ponta Grossa - Guarapuava, on living leaves of *Conyza canadensis*, 12 october 2010, E. Guatimosim (VIC 31687). Brazil, state of Paraná, Laranjal, on living leaves of *Conyza canadensis*, 13 october 2010, E. Guatimosim (VIC 31686). Brazil, state of Paraná, Janiópolis, on living leaves of *Conyza canadensis*, 14 october 2010, E. Guatimosim (VIC 31608). Brazil, state of Rio Grande do Sul, Nova Petropolis, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31632). Brazil, state of Paraná, Capivari – Vale do Sonho, on living leaves of *Conyza canadensis*, 17 october 2010, E. Guatimosim (VIC 31622). Brazil, state of Paraná, Colombo – Vale do Sonho, on living leaves of *Conyza canadensis*, 19 october 2010, E. Guatimosim (VIC 31613). Brazil, state of Minas Gerais, Unaí – Cristalina, on living leaves of *Conyza canadensis*, 28 November 2010, L. L. Duarte, (VIC 31698). Brazil, state of Goias, Santa Barbára do Goias, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31699). Brazil, state of Goias, Goiania, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31700). Brazil, state of Goias, Goiania, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31638). Brazil, state of Goias, Goiania, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31701). Brazil, state of Goias, Santa Barbára do Goias, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31702). Brazil, state of Goias, Anápolis, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31703). Brazil, state of Goias, Inaciolândia - Itubiara, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31696). Brazil, state of Goias, Inaciolândia - Itubiara, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31697). Brazil, state of Goias, Rio Verde, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31640). Brazil, state of Goias, Jatai - Rio Verde, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC). Brazil, state of Goias, Pantaninho on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31606). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31705). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31706). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31707). Brazil, state of Minas Gerais, São Gotardo, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31694). Brazil, state of Minas Gerais, Coromandel - Patrocinio, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31605). Brazil, state of Minas Gerais, Patrocinio - Ibiá, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31693). Brazil, state of Minas Gerais, Ibiá - São Gotardo, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC31695). Brazil, state of Minas Gerais, Patrocinio, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31704).

Notes: Powdery mildews are common and easily recognizable diseases of plants, all of which are caused by members of the Erysiphaceae (Agrios, 2005). *Podosphaera fusca* (Fr.) Blumer, emend. Braun is considered to be the most

commom and polyphagous species of the family with a very broad host range. This species is described on different hosts genera of the subclass Asteridae (Braun, 1995), including *C.canadensis* where it was recorded many times in several contries (Farr *et al*., 2011). Strangely, until now, there was not a single record of this association in the Americas, which is probably due to the lack of surveys of fungi associated with this weed. All features of the fungi collected on *C. canadensis* are equivalent to those described for *P. fusca* by Braun with the exception that the conidiophores are 30 µm longer. It is a well known fact that the morphological characters of Erysiphaceae are plastic (Pérez-García *et al*., 2009) and that temperature, humidity and other factors may influence the length of the conidiophores (Braun, 1987) which lead to the fact that the conidiophores length can not be used as marker to separation between species. Thus, we decided that the fungus on *C. canadensis* fits well with the anamorphic state of *P. fusca*. This is the first report of *P. fusca* parasiting the weed *C.canadensis* in the new world.

This was one of the most common fungi found in association with *C. canadensis* in the present study being recordedin almost all samples points. Although ubiquitous this fungus was only seen causing moderate, and seemingly not significantly debilitating, disease symptoms on its host. As it also has a world distribution on this host, plus a wide host range, it is regarded here as not having potential for use in classical biological control. As fungi in the Erysiphales are well known biotrophs they are naturally regarded as having no potential as mycoherbicides.

Septoria erigerontis Peck, *Annual Report on the New York State Museum of Natural History* 24: 87 (1872); Fig. 18

Lesions on living leaves concentric, initially circular to sub circular and elliptic when older, adaxially black with a grayish and depressed center with dark brown edges, abaxially dark brown, 1.0–8.0 mm diam. External mycelium absent. Internal mycelium intercelular, up to 3.0 µm diam, branched, cylindrical, septate, hyaline to sub-hyaline. Conidioma pycnidial, amphigenous but more abundant adaxially, immersed then erumpent, solitary, randomly distributed over the injured tissue, globose to sub globose, walls of textura angularis,unilocular, 91.5–150.0 ×

76.5–133.5 µm, 1–2 cells thick, 7.0–9.5.0 µm, brown becoming dark brown near the ostiole, smooth. Deiscence ostiolar, central, one per conidioma, 9.0–20.0 µm. Conidiophores often reduced to conidiogenous cells, cylindrical, straight, 7.5–19.5 × 2.5–10.0 µm, hyaline to sub-hyaline, smooth. Conidiogenous cells holoblastic, obpyriform, 4.5–10.0 × 2.0–10.0 µm, hyaline to sub hyaline, smooth. Conidia filiform, curved, 36.0–55.0 × 2.0–2.5 µm, 1–5 septate, minutely gutulate, hyaline to sub-hyaline, smooth.

In culture: slow-growing (1.3 cm diam in 10 days on PCA, 1.5 cm in 10 days on PDA), colony flat of sparse cottony aerial mycelium, moist, mouse grey on PCA; On PDA stromatic, irregularly raised (sub-cerebriform) with some buff aerial mycelium centrally and cottony to felty isabelline mycelium at periphery, compressing the medium; no sporulation. Reverse: On both PCA and PDA dark mouse grey with the periphery Isabelline (PCA) or honey (PDA).

Sequences: Comparison of the rDNA internal transcribed spacer (ITS) sequence with the available sequences in the GenBank showed a 100% of nucleotide identity (99% of query coverage) with the specie *Septoria erigerontis* (GenBank accession number GU952666).

Material examined: Brazil, state of Minas Gerais, São João Del Rey - Tiradentes, on living stems of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31620). Brazil, state of Minas Gerais, Pouso Alegre - Poços de Caldas, on living stems of *Conyza canadensis*, 21 April 2010, E. Guatimosim (VIC 31655). Brazil, state of São Paulo, Aguas da Prata, on living leaves of *Conyza canadensis*, 22 April 2010, E. Guatimosim (VIC 31615). Brazil, state of São Paulo, Limeira, on living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31656). Brazil, state of São Paulo, Mairiporã, on living leaves of *Conyza canadensis*, 23 April 2010, E. Guatimosim (VIC 31618). Brazil, state of Minas Gerais, São Geraldo, on living leaves of *Conyza canadensis*, 23 June 2010, L. L. Duarte, (VIC 31661). Brazil, state of Paraná, Ponta Grossa - Guarapuava, on living leaves of *Conyza canadensis*, 12 october 2010, E. Guatimosim (VIC 31628). Brazil, state of Paraná, Capivari, on living stems of *Conyza canadensis*, 12 october 2010, E. Guatimosim (VIC 31657). Brazil, state of Rio Grande do Sul, Marau, on living leaves of *Conyza canadensis*, 15 October 2010, E. Guatimosim (VIC 31611). Brazil, state of Paraná, Concordia, on living leaves of *Conyza canadensis*, 15 october 2010, E. Guatimosim (VIC 31624). Brazil, state of Paraná, Capivari – Vale do Sonho, on living leaves of *Conyza canadensis*, 17 october 2010, E. Guatimosim (VIC 31622). Brazil, state of Santa Catarina, Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31635). Brazil, state of Goias, Goiania, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31638). Brazil, state of Goias, Paragominas, on living leaves of *Conyza canadensis*, 04 December 2010, L. L. Duarte, (VIC 31663). Brazil, state of Goias, Quirinopolis – Gouvelandia, on

living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31642). Brazil, state of Goias, Cachoeira Dorada, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31662). Brazil, state of Goias, Santa Helena - Quirinópolis, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31637). Brazil, state of Goias, Jatai - Rio Verde, on living leaves of *Conyza canadensis*, 05 December 2010, L. L. Duarte, (VIC 31667). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31665). Brazil, state of Minas Gerais, Uberaba, on living leaves of *Conyza canadensis*, 06 December 2010, L. L. Duarte, (VIC 31666). Brazil, state of Minas Gerais, Ibiá, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31639). Brazil, state of Minas Gerais, Coromandel - Patrocinio, on living leaves of *Conyza canadensis*, 07 December 2010, L. L. Duarte, (VIC 31605).

Notes: *Septoria* is one of the largest genera of anamorphic fungi including more than 1.000 species, most of which are plant parasites (Kirk *et al*., 2008). Mostly were described as separate species based on supposed host-specificity only and their distinction as separate species was doubted by several authors (Priest, 2006; Jorstad, 1965; cited by Verkley *et al*., 2004). However, ongoing studies are surprisingly confirming that most species erected on host basis only are in fact distinct (P. Crous, pers. comm.) Approximately 200 are parasitic on members of the Asteraceae. Only *S. erigerontis* was recorded in association with *C. canadensis* (Farr *et al*., 2011). The main morphological characters used in the modern taxonomy of *Septoria* spp. are conidial shape, length, width and septation (Shin *et al*, 2001) and the conidial features, including morphometric data of our samples fits well with the description of *S. erigerontis* and our molecular data reinforces it. This is the first report of *S. erigerontis* in association with *C. canadensis* in Brazil.

This species was frequently found in all Brazilian regions covered during the survey.

Five days after inoculation, the first symptoms observed were small spots surrounded by a chlorotic halo that quickly led to the formation of a necrotic area (still surrounded by a chorotic halo but now larger with a grey center where it was possible to visualize small black dots - the pycnidia. In the field conditions it caused small necrotic lesions on leaves of *C. canadensis* and wasn't particularly damaging to the host. Nevertheless damage was much more severe in artificial inoculations with mycelium plugs. It appears that, in case an effective method for mass-production of virulent inoculum is developed, this fungus may be suitable for mycoherbicide development.

Sphaerulina sp. nov. Fig. 19

Lesions on living leaves amphigenous, irregular, covering part of the leaf, dark brown, with fruting bodies scattered randomly under the injured tissue on both sides of the leaves. External mycelium absent. Internal mycelium intercellular, 2.0–3.0 µm diam, subcylindrical, branched, septate, subhyaline. Ascomata pseudotecial, adaxial, subcuticular to subepidermal, solitary, subglobose, papilate, walls of textura angularis, 59.0–148.5 × 87.0– 160.0 µm, 2 cells thick, 4.0–10.0 µm, brown. Deiscence ostiolate, central, one per ascoma, circular, 20.5–28.0 µm diam. Lack of interthecial filament. Asci bitunicate, fasciculate, obclavate, 39.5–65.5 × 7.5–10.5 µm, 8-spored. Ascospores fusiform, slightly curved, 18.0–27.0 × 3.5–4.0 µm, inordinate, 3–7 septate, gutulate, hyaline, smooth.

Material examined: Brazil, state of Santa Catarina, Iaguará do Sul, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31836). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634).

Notes: The genus *Sphaerulina* was first described by Saccardo (1878) who used ascospore septation as the key character for separating it from *Mycosphaerella*-like species as well as other fungi belonging to Dothideales. Crous *et al*. (2003) raised doubts, based on their partial phylogenetic study of *Sphaerulina* and related genera, about the value of ascospore septation for separation between genera and questioned the taxonomic validity of *Sphaerulina*. But no further detailed and definitive study was conducted and the genus remains valid although possibly artificial. Attempts to culture this fungus were unsuccessful rendering molecular studies difficult to perform at this stage. Therefore it was decided that the traditional Saccardo treatment should be used for the fungus on *C. canadensis* and we chose to place it in *Sphaerulina*. Despite the large number of described species in this genus none is present in association with any Asteraceae host, and so, considering a purported host-specificity of this fungus it was regarded as a new species for the genus.

This fungus has its distributuion very restricted being collected only twice during this survey in regions very close to each other in the Santa Catarina state. It

was capable of causing extensive leaf necrosis, but as no cultures of this fungus were obtained it is not possible to conjecture, at this stage, about its potential as a biocontrol agent. This is the first report of a *Sphaerulina* sp. on a member of *Conyza* worldwide

Wentiomyces melioloides (Berkeley & M.A. Curtis) E. Müller, *Beiträge zur Kryptogamenflora der Schweiz* 11(2): 493 (1962); Fig. 20

Colonies on living leaves, adaxial, sooty, circular to irregular, sometimes leading to slight yellowing of tissues underneath and sometimes become purplish at the edges of colonies and necrotic abaxially, 1.0–3.5 mm diam. External mycelium forming a subiculum, branched,1.5–2.5 µm diam, septate, medium brown to sub-hyaline, smooth and ending as appressoria or penetrating through stomata. Appressoria round to sub-round shaped, straight, 5.0–9.0 µm diam, sub-hyaline. Internal mycelium intercellular, 2.0–3.0 µm diam, sparingly branched, cylindrical, septate, hyaline. Ascomata pseudotecial, epiphyllous, superficial, scattered irregularly over the whole surface of the colony, spherical to oblate spheroidal and somewhat flattened, 49.0–65.0 × 56.0–93.5 µm, walls of textura angularis, 3 cells, 5.0–9.0 µm thick, dark brown, smooth. Setae arising from the upper third of the pseudothecia circling the entire perimeter of the ascoma, cylindrical straight to slightly curved,19.0–99.0 × 3.0–4.0 multiseptate (up to 6), smooth, light golden brown, unbranched, with rounded tips. Deiscence ostiolar, central, one per ascoma, oval to circular, papillate or not, 9.0–25.5 µm diam. Interthecial filament type paraphyisoid, filiform, 0.5–1.0 µm diam, septate, unbranched, hyaline. Asci parallel, cylindrical to obclavate, 23.0–36.0 × 6.5–9 µm, 8-spored. Ascospores ellipsoid to slightly obclavate, with acute to rounded ends, 7.5–9.0 (–12.0) × 2.5–4.0 µm, inordinate, 1-septate, rarely 2-septate, when 1-septated the septum is near the middle making the upper cell to be slightly bigger, gutulate but the gutules disappear when older,hyaline becoming light brown when mature, smooth and slightly verruculose when older.

Material examined: Brazil, state of Paraná, Ponta Grossa - Guarapuava, on living leaves of *Conyza canadensis*, 12 october 2010, E. Guatimosim (VIC 31628). Brazil, state of Rio Grande do Sul, Iaquara, on living leaves of *Conyza canadensis*, 16 October 2010, E. Guatimosim (VIC 31621). Brazil, state of Santa Catarina, Urussanga, on living leaves of *Conyza canadensis*, 17 October 2010,

E. Guatimosim (VIC 31623). Brazil, state of Santa Catarina, São Francisco do Itaperiu – Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31634). Brazil, state of Santa Catarina, Massaranduba, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31635). Brazil, state of Santa Catarina, São Francisco do Itaperiu, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31647). Brazil, state of Santa Catarina, Iaguará do Sul, on living leaves of *Conyza canadensis*, 18 October 2010, E. Guatimosim (VIC 31636).

Notes: Initially this fungus was considered as possibly belonging to either the genus *Eumela* or to *Wentiomyces.* Observation of presence or absence of stomatopodia, the only distinguishing feature for these two genera (present in *Eumela* and absent in *Wentiomyces* according to von Arx & Muller (1975)), was challenging. More careful observations led to the final conclusion that the fungus found in the survey actually belonged to *Wentiomyces*.

This taxon was first described by Koorders in 1907 with *W. javanicus* as the type species. There are about 17 species accepted within this genus but only three are reported in association with members of the Asteraceae. These are: *W. clavisetus* (Doidge) Arx (on *Erlangea marginata* S. Moore), *W. fimbriatus* (Dearn. & House) M.E. Barr (on *Achillea millefolium* L.) and *W. melioloides* (Berk. & M.A. Curtis) E. Müll (on *Laginifera* spp.). The fungus found during the survey has a morphology which is in all equivalent to that described for *W. melioloides* with few exceptions: ascospore surface which is slightly verruculose in older spores, guttules that disappear in mature ascospores and the yellowing and necrosis of colonized tissues in older lesions (features not mentioned in Muller & von Arx, 1962), but as no significant morphological differences were found between the newly collected material and *W. melioloides* we decided to place it within this taxon. Therefore this is the first record of *W. melioloides* on a member of the genus *Conyza.*

This fungus was only found in southern Brazil (although it occurs in all states of this region) indicating that *W. melioloides* may be adapted to cooler climates. Fungi in this group are biotrophic, and host-damage is minimal. We regard this fungus as of no use for weed biocontrol.

Discussion

There are numerous reports of nearly fifty fungal species parasitizing the weed *Conyza canadensis* throughout the world (Table 4). This was to be expected for

such a cosmopolitan weed and is similar to what has been found for other weeds such as *Chromolaena odorata* (Barreto & Evans, 1994), *Mikania micrantha* Barreto & Evans (1995), *Lantana camara* (Barreto *et al*., 1995) and many other weeds for which literature surveys of known mycobiota, aimed at biological control, have been performed. Nevertheless, this is the first time that a literature and field survey for fungi on this important host has been performed. An examination of Table 4 suggests that many of the fungal pathogens of *C. canadensis* have been distributed along with its host as it spread from the Americas into the old world. One important exception appears to be *Puccinia asterum* (Schwein.) F. Kern, which appears to have remained restricted to the new world, perhaps even to a restricted area in North America. Little is known about this fungus but rust fungi have repeatedly proven to be useful classical biocontrol agents such as *Puccinia chondrillina* Bubák & Syd. to control *Chondrilla juncea* L. (Supkoff *et al*., 1988; Charudattan & Dinoor, 2000) and *Maravalia cryptostegiae* (Cummins) Y. Ono to control *Cryptostegia grandiflora* Roxb. ex R. Br. (Evans & Tomley, 1994; Tomley & Evans, 2004) and a rust associated to such an important agricultural weed should not be neglected by biocontrol workers. Two somewhat strange records found in the literature of fungi on *C. canadensis* are those of *Entyloma compositarum* Farl. (from former Czechoslovakia) and *Entyloma erigerontis* Syd. & P. Syd. ex Cif (from Germany). Fungi in this genus are known to be highly specialized host-specific fungal pathogens causing white smut. One species belonging to this genus has been successfully used as a classical biocontrol agent agains *Ageratina riparia* in the USA (Hawaii) Barreto & Evans (1988) and New Zealand (Barton *et al*., 2007). It appears strange that such specialized pathogens have appeared on *C. canadensis* in Europe but are not known in the native range of the weed in the new world. Perhaps this is explained by lack of intensive surveys on this weed in North America.

The pioneering field survey of fungal pathogens of *C. canadensis* performed in Brazil, presented here, indicates that there may be potential for classical introduction of fungi from North America, several of which (including *P. astereum*) did not appear during the survey and which may prove to be adequate candidates (if sufficiently damaging to the host and safely host-specific) for introduction into Brazil. None of the fungi collected in Brazil appeared to be particularly useful for use in classical biological control elsewhere. The only obvious candidate would be the

oomycete *B. entospora* but judging from the published records the species is already widespread and its impact on the weed populations appears to have been insufficient for its control. Preliminary studies undertaken here suggested that one species collected in the survey appeared sufficiently damaging to be further investigated as a mycoherbicide - *Phoma canadensis*. It was fast growing in culture and capable of significantly reducing growth and causing plant death in inundative applications. It is clearly worthy of further investigations in the future as a potential bioherbicide for *C. canadensis*.

Twelve fungal species were collected on *C. canadensis* during the survey in Brazil and were described. These included three new taxa, which were proposed herein. With the except of *Cercosporella virgaureae*, there was no previous published record of fungi on *C. canadensis* in Brazil, and all the fungal taxa recorded here represent new hosts records or new records of fungi for Brazil.

The mycobiota described herein may represent only a fraction of the existing diversity of fungal pathogens on this host as only part of the distribution range of this plant in the new world was covered during the survey. It is likely that an expansion of the survey, including areas in Central and North America will probably significantly increase the list of fungi associated with *C. canadensis* maybe uncovering fungi with even higher biocontrol potential than those described here.

Acknowledgements

This work forms part of a research project submitted as a M.Sc. dissertation to the Departamento de Fitopatologia/Universidade Federal de Viçosa by L.L. Duarte. The authors thanks Dr. Uwe Braun and Anibal Alves de Carvalho Júnior for help in the identication of *Podosphaera fusca* and *Aecidium* sp. nov., respectively, and to Braz Hora Júnior for help in bioinformatics. The authors also thank CNPq, CAPES and FAPEMIG for financial support.

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Table 1. List of *Cercospora* isolates and the outgroups included in the study, with their country of origin and host

Strain	Host	Origin	GenBank number		
			ITS	TEF	CAL
Cercospora acaciae-mangii					
CPC 10527	*Acacia* sp.	Australian	AY752142	AY752177	AY752236
CPC 10526	*Acacia* sp.	Australian	AY752141	AY752176	AY752235
CPC 10553	*Acacia* sp.	Australian	...	AY752175	AY752234
CPC 10552	*Acacia* sp.	Australian	...	AY752174	AY752233
CPC 10551	*Acacia* sp.	Australian	AY752140	AY752173	AY752232
CPC 10550	*Acacia* sp.	Australian	AY752139	AY752172	AY752231
Cercospora apii					
CBS 119.25; CPC 5086	*Apium graveolens*	...	AY840512	AY840479	AY840410
CBS 121.31; CPC 5073	*Beta vulgaris*	Austria	AY840513	AY840480	AY840411
CBS 127.31; CPC 5119	*B. vulgaris*	Hungary	AY840514	AY840481	AY840412
CBS 152.52; CPC 5063	*B. vulgaris*	Netherlands	AY840515	AY840482	AY840413
CBS 536.71; CPC 5087	*A. graveolens*	Romania	AY752133	AY752166	AY752225
CBS 114416; CPC 10925	*Apium* sp.	Austria	AY840516	AY840483	AY840414
CBS 114418; CPC 10924	*A. graveolens*	Italy	AY840517	AY840484	AY840415
CBS 114485; CPC 10923	*A. graveolens*	Italy	AY840518	AY840485	AY840416
CBS 116455; CPC 11556	*A. graveolens*	Germany	AY840519	AY840486	AY840417
CBS 116504; CPC 11579	*A. graveolens*	Germany	AY840520	AY840487	AY840418
CBS 116507; CPC 11582	*A. graveolens*	Germany	...	AY840488	AY840419
Cercospora apiicola					
CBS 116457; CPC 10267	*Apium* sp.	Venezuela	...	AY840503	AY840434
CBS 116458; CPC 10657	*Apium* sp.	Korea	...	AY840504	AY840435
CPC 10220	*Apium* sp.	Venezuela	...	AY840505	AY840436
CPC 10248	*Apium* sp.	Venezuela	...	AY840506	AY840437
CPC 10265	*Apium* sp.	Venezuela	...	AY840507	AY840438
CPC 10266	*Apium* sp.	Venezuela	AY840541	AY840508	AY840439
CPC 10279	*Apium* sp.	Venezuela	AY840542	AY840509	AY840440
CPC 10666	*Apium* sp.	Korea	AY840543	AY840510	AY840441
CPC 10759	*A. graveolens*	Korea	AY840544	...	AY840442
CPC 11642	*Apium* sp.	Greece	DQ233341	...	...
CPC 11641	*Apium* sp.	Greece	DQ233340	...	...
Cercospora beticola					
CBS 116.47; CPC 5074	*B. vulgaris*	Netherlands	AY752135	AY752168	AY752227
CBS 122.31; CPC 5072	*B. vulgaris*	Germany	AY752136	AY752169	AY752228
CBS 123.31; CPC 5071	*B. vulgaris*	Spain	AY840522	AY840489	AY840420
CBS 124.31; CPC 5070	*B. vulgaris*	Romania	AY840523	AY840490	AY840421
CBS 125.31; CPC 5069	*B. vulgaris*	Japan	AY840524	AY840491	AY840422
CBS 126.31; CPC 5064	*B. vulgaris*	Germany	AY840525	AY840492	AY840423
CBS 116454; CPC 11558	*B. vulgaris*	Germany	AY840526	AY840493	AY840424
CBS 116456; CPC 11557	*B. vulgaris*	Italy	...	AY840494	AY840425
CBS 116501; CPC 11576	*B. vulgaris*	Iran	AY840528	AY840495	AY840426
CBS 116502; CPC 11577	*B. vulgaris*	Germany	AY840529	AY840496	AY840427
CBS 116503; CPC 11578	*B. vulgaris*	Italy	AY840530	AY840497	AY840428
CBS 116505; CPC 11580	*B. vulgaris*	France	AY840531	AY840498	AY840429
CBS 116506; CPC 11581	*B. vulgaris*	Netherlands	AY840532	AY840499	AY840430
CPC 5125	*B. vulgaris*	New Zealand	AY752137	AY752170	AY752229
CPC 5128	*B. vulgaris*	New Zealand	AY752138	AY752171	AY752230
CPC 10168	*B. vulgaris*	New Zealand	AY840533	...	AY840431
CPC 10171	*B. vulgaris*	New Zealand	AY840534	AY840501	AY840432
CPC 10197	*B. vulgaris*	New Zealand	AY840535	AY840502	AY840433
Cercospora canescens					
CPC 4409	*Citrus paradisii*	South Africa	...	DQ835087	DQ835133
CPC 4408	*Citrus paradisii*	South Africa	...	DQ835086	DQ835132
CBS 111134; CPC 1138	*Vigna* sp.	South Africa	...	DQ835085	DQ835131
CBS 111133; CPC 1137	*Vigna* sp.	South Africa	...	DQ835084	DQ835130
CBS153.55	...	...	AF163086	...	...
CCA19	...	...	AY266164	...	...
STE-U 1137	...	...	AY260065	...	...
STE-U 1138	...	...	AY260066	...	...
CBS 135.28; CPC 5067	*Glycine* sp.	Japan	DQ835071	DQ835089	DQ835135
CBS 128.27; CPC 5068	*Glycine* sp.	Japan	DQ835070	DQ835088	DQ835134

To be continued...

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Strain	Host	Origin	GenBank number		
			ITS	TEF	CAL
Cercospora physalidis					
CBS 570.69; CPC 5075	*Nicotiana tabacum*	...	DQ835074	DQ835100	DQ835147
CBS 131.32; CPC 5076	*Nicotiana tabacum*	...	DQ835073	DQ835099	DQ835146
Cercospora piaropi					
CBS 113127; TX-18	*Eichhornia crassipes*	...	DQ835075	DQ835101	DQ835148
FJ24	...	China	HQ902254	...	...
Cercospora rodmanii					
15-GTOX	...	Mexico	GQ884185	...	GQ884195
5H-GTOX	...	Mexico	GQ884184	...	GQ884194
***Cercospora* sp.**					
RWB 968f	*Conyza. canadensis*	Brazil			
Mycosphaerella thailandica					
CPC 10549	*Acacia* sp.	Australian	AY752158	AY840478	AY752250
CPC 10548	*Acacia* sp.	Australian	AY752157	AY840477	AY752249
CBS 116367; CPC 10547	*Acacia* sp.	Australian	AY752156	AY840476	AY752248

Table 2. List of strains included in analyses of *Cercosporella virgaureae,* with their country of origin and host

Strain	Host	Origin	GenBank number
Cercospora apii			
CBS:118712	...	Fiji	GQ852583
Cercospora zebrinae			
CBS:118790	*Trifolium subterraneum*	Australia	GQ852584
Cercosporella centaureicola			
CBS 120253	*Centaurea solstitialis*	Greece	EU019257
Cercosporella virgaureae			
CBS:113304	*Erigeron annuus*	South Korea	GQ852585
BSV 09	*Conyza canadensis*	Brazil	
Mycosphaerella acaciigena			
CBS:112515; CPC:3837	*Acacia mangium*	Venezuela	GQ852599
CBS:112516; CPC:3838	*Acacia mangium*	Venezuela	GQ852600
Mycosphaerella bixae			
CBS:111804; CPC:2554	*Bixa orellana*	Brazil	GQ852630
Mycosphaerella heimii			
CBS:110682; CPC:760	*Eucalyptus* sp.	Madagascar	GQ852604
CPC:11000	*Eucalyptus* sp.	Colombia	GQ852605
CPC:13099	*Eucalyptus dunnii*	Australia	GQ852606
Mycosphaerella heimioides			
CBS:111190; CPC:1312	*Eucalyptus* sp.	Indonesia	GQ852607
Mycosphaerella holualoana			
CBS:110699; CPC:2155	*Leucospermum* sp.	USA:Hawaii	GQ852608
Mycosphaerella irregulariramosa			
CBS:111211; CPC:1362	*Eucalyptus saligna*	South Africa	GQ852609
Mycosphaerella keniensis			
CBS:111001; CPC:1084	*Eucalyptus grandis*	Kenya	GQ852610
Mycosphaerella konae			
CPC:10992	*Eucalyptus* sp.	Colombia	GQ852611
Mycosphaerella marksii			
CBS:110942; CPC:982	*Eucalyptus botryoides*	Australia	GQ852612
Passalora ageratinae			
CPC 15365; CBS:125419	*Ageratina adenophora*	South Africa	GU214453
Passalora intermedia			
CBS 124154; A39; CPC 15745	*Eucalyptus camaldulensis*	Madagascar	FJ790297
CPC 15733; A26	*Eucalyptus camaldulensis*	Madagascar	FJ790295
CPC 15737; A30	*Eucalyptus camaldulensis*	Madagascar	FJ790296
Phoma medicaginis			
CZ509-1	...	China	FJ755251
Pseudocercosporella bakeri			
CPC:17570	*Ipomoea* sp.	Philippines	GU570553
Pseudocercospora basitruncata			
CBS 111280	*Eucalyptus* sp.	Thailand	DQ204760
CBS 114665	*Eucalyptus* sp.	Thailand	DQ204759
Pseudocercospora crousii			
CBS:119487	*Eucalyptus* sp.	New Zealand	GQ852631
Pseudocercospora griseola f. griseola			
CPC 10461			GU348997
CPC:10779	*Phaseolus vulgaris*	South Korea	GQ852633
Pseudocercospora sphaerulinae			
CBS:112621; CPC:4314	*Eucalyptus* sp.	Chile	GQ852652

To be continued...

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Strain	Host	Origin	GenBank number
Ramularia aplospora			
CBS 545.82	...	Germany	EU040238
Ramularia miae			
CBS 120121; CPC 12736	*Wachendorfia thyrsifolia*	South Africa	DQ885902
Ramularia pratensis var. pratensis			
CPC 11294	*Rumex crispus*	South Korea	EU019284
Ramulispora sorghi			
CBS:110578; CPC:905	*Sorghum* sp.	South Africa	GQ852653
Teratosphaeria cryptica			
CPC:12415	*Eucalyptus globulus*	Australia	GQ852683
CPC:12424	*Eucalyptus globulus*	Australia	GQ852684
CPC:12559	*Eucalyptus nitens*	Australia	GQ852685
CPC:12562	*Eucalyptus nitida*	Australia	GQ852686
CPC:12565	*Eucalyptus amygdalina*	...	GQ852687
CPC:13839	*Eucalyptus globulus*	Australia	GQ852688
Teratosphaeria nubilosa			
CPC:11926	*Acacia auriculiformis*	Thailand	GQ852694
CPC:12235	*Eucalyptus globulus*	Portugal	GQ852695
CPC:12243	*Eucalyptus globulus*	Portugal	GQ852696
CPC:12830	*Eucalyptus globulus*	Portugal	GQ852697
CPC:13452	*Eucalyptus sp.*	Portugal	GQ852698
CPC:13825	*Eucalyptus globulus*	Australia	GQ852699
Teratosphaeria parva			
CPC:12249	*Eucalyptus globulus*	Portugal	GQ852708
CPC:12419	*Eucalyptus globulus*	Australia	GQ852709

Table 3. *Aecidium* or aecidial stages recorded on *Conyza* spp. hosts worldwidely.

Fungi	Host	Position	Sperm*	Aecia (µm diam)	Peridium cells		Aeciospores		
					Shape	Size (µm)	Shape	Color	Size (µm)
Aecidium sp. nov.	*C. canadensis* (L.) Cronq.	Hypophylous	V	135.0–218.5	Polyhedral, sub rhomboid to sub rectangular	10.5–24.5 × 11.5– 25.5	Subglobose to slightly ellipsoid	Hyaline with orange content	14.0–24.5 × 13.0–19.5
Aecidium conyzae-colombiensis Pardo-Cardona	*C. bonariensis* (L.) Cronq.	Amphigenous	...	210.0–260.0	Oval, rhomboid to sub rhomboides	25.0–37.5 × 17.5–20.0	Globose to subglobose	Subhyaline	12.0–31.0 × 13.0–18.0
Aecidium conyzae-pinnatilobatae P. Syd. & Syd.	*C. pinnatilobata* DC.	Hypophylous	...	250.0–300.0	Subrhomboid	20.0–28.0 × 16.0–20.0	Globose to ellipsoid	Subhyaline	15.0–19.0 × 13.0–17.0
Aecidium hoffmannii P. Syd. & Syd.	*C. limosa* O. Hoffm.	Hypophylous	...	250.0	Subrhomboid	25.0–36.0 × 14.0–18.0	Globose to ellipsoid	Subhyaline	17.0–23.0 × 13.0–17.0
Aecidium spegazzinii De Toni	*C. bonariensis* (L.) Cronq.	Amphigenous	...	150.0–180.0	Obvoid to ellipsoid-rhomboid	18.0–20.0 × 12.0–16.0	Subglobose	Hyaline	14.0–17.0 × 12–15
Aecidium niederleinii Henn.	*C. sinensis* J.F.Gmel.	Hypophylous	...	...	Orbicular	20.0–35.0 × 15.0–23.0	Subglobose to ovoid	Yellow-brown	15.0–18.0 × 14.0–17.0
Puccinia alia H.S. Jacks. & Holw.	*C. trinervis* Lam.	Amphigenous	...	...	Lack		Ellipsoid or obvoid	...	(23.0–)26.0–35.0 × (16.0) 18.0–23.0(–25.0)
Puccinia cyperi Arthur	*C. bonariensis* (L.) Cronq.	Hypophylous	...	...	...	...	Obvoid or ellipsoid	...	21.0–31.0 × 15.0–21.0
Puccinia dioicae Magnus	*C. canadensis* (L.) Cronq.	Hypophylous	Indistinct	...	...	...	Subglobose	...	12.0–15.0

*spermogonia

Table 4. Fungi recorded on the weed *Conyza canadensis* worldwide*

Fungi	Distribution
Pezizomycetes and anamorphic fungi	
Alternaria sp.	USA (Roy *et al*.,1994)
Alternaria tenuissima (Nees) Wiltshire	China (Zhang, 2003)
Cercosporella virgaureae (Thüm.) Allesch	Canada (Ginns,1986); Austria, Belgium, Bulgaria, Canada, Caucasus, France, Germany, Hungary, Illinois, Italy, Kazakhstan, Kyrgyzstan, USA, Poland, Romania, Russia, Spain, Taiwan, Ukraine (Braun, 1995a); Korea (Cho& Shin, 2004)
Clathrospora diplospora (Ellis & Everh.) Wehm.	Poland (Adamska, 2001)
Colletotrichum sp.	USA (Roy, 1994); Canada (Weaver, 2001)
Crocicreas nigrescens (Rehm) S.E. Carp	USA (Carpenter, 1981); Canada (Weaver, 2001)
Diaporthopsis apiculosa (Ellis) Wehm	USA (Cash,1952); Canada (Weaver, 2001)
Diaporthe arctii (Lasch) Nitschke.	USA (Anonymous, 1960); Canada (Weaver, 2001)
Didymosphaeria epidermidis (Fr.) Fuckel	USA (Hanlin,1963)
Diplodina erigerontis Hollós	West Indies (Minter et al., 2001); Dominican Republic (Ciferri, 1961)
Erysiphe cichoracearum DC.	USA (Shaw, 1973); Czechoslovakia, Romania and Russia (Braun, 1995b); Germany (Ali et al., 2000); Poland (Czerniawska, 2001)
Fusarium sp.	Poland (Adamska, 2001)
Fusarium oxysporum Schltdl	USA (Helbig & Carroll, 1984)
Heptameria obesa (Durieu & Mont.) Sacc	USA (Hanlin, 1963)
Hypoderma commune (Fr.) Duby	USA (Hanlin, 1963)
Lasiostemma melioloides (Berk. & Ravenel) Theiss	Dominican Republic (Ciferri, 1961)
Leptosphaeria canadensis De No	Canada and Italy (Crane &Shearer, 1991);
Leptosphaeria congesta M.T. Lucas	Portugal (Sivanesan, 1984); Spain (Camara,2002)
Leptosphaeria erigerontis Berl	USA (Shoemaker, 1984); Canada (Weaver, 2001)

To be continued…

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Fungi	Distribution
Pezizomycetes and anamorphic fungi	
Leptosphaeria longipedicellata J.H. Mill. & Burton	USA (Hanlin, 1963)
Leptosphaeria ogilviensis (Berk. & Broome) Ces. & De Not.	Poland (Mulenko *et al*., 2008)
Macrophomina phaseolina (Tassi) Goid	USA (Anonymous,1960); Canada (Weaver, 2001)
Mollisia atrata Bres	USA (Hanlin,1963)
Mollisia exígua (Cooke) Seaver	USA (Dennis, 1964); Canada (Weaver, 2001)
Neoerysiphe cumminsiana (U. Braun) U. Braun	India (Baiswar, 2008)
Oidium sp.	Russia (Rusanov & Bulgakov, 2008)
Phoma canadensis Allesch	USA (French, 1989)
Phomopsis sp.	USA (Roy et al., 1994)
Phymatotrichopsis omnivora (Duggar) Hennebert	USA (Anonymous, 1960)
Pleospora herbarum (Pers.) Rabenh	Poland (Mulenko et al., 2008)
Podosphaera fuliginea (Schltdl.) U. Braun & S. Takam	Canada (Ginns, 1986); Central Asia (Koshkelova & Frolov, 1973); Finland (Kari,1957); Portugal (Unamuno,1941); USA (Anonymous,1960)
Podosphaera fusca (Fr.) U. Braun & Shishkoff	Belarus (Girilovich et al., 2005); Bulgaria (Fakirova,1991); China (Park et al., 2010); Germany (Ali et al.,2000); Iraq (Amano, 1986); Korea (Shin, 2000); Poland (Majewski, 1971); Russia (Rusanov & Bulgakov, 2008); Switzerland (Bolay, 2005), Turkey (Karakaya,1998); Yugoslavia (Rankovic & Comic,1997)
Podosphaera macularis (Wallr.) U. Braun & S. Takam.	USA (Shaw, 1973); Canada (Weaver, 2001)
Podosphaera sp.	Japan (Ito &Takamatsu, 2010)
Pyrenophora penicillus (J.C. Schmidt ex Fr.) Sacc.	Germany (Shoemaker,1992)
Ramularia macrospora Fresen.	USA (Shaw, 1973)
Sclerotium rolfsii Sacc.	USA (Alfieri et al., 1984)
Septoria erigerontis Peck	Canada (Weaver, 2001); Korea, Portugal Turkey (Erper et al., 2010); USA (Anonymous, 1960)

To be continued…

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Fungi	Distribution
Pezizomycetes and anamorphic fungi	
Stictis radiata (L.) Pers	USA (Hanlin, 1963)
Synchytrium aureum J. Schröt	Germany and Poland (Karling, 1964);
Synchytrium macrosporum Karling	USA (Walker,1983)
Thielaviopsis basicola (Berk. & Broome) Ferraris	Canada (Ginns, 1986)
Basidiomycetes	
Entyloma compositarum Farl.	Czechoslovakia (Zundel, 1953)
Entyloma erigerontis Syd. & P. Syd. ex Cif	Germany (Scholz &Scholz, 1988)
Puccinia asterum (Schwein.) F. Kern	USA (Hunt,1926)
Puccinia cyperi Arthur	USA (Anonymous,1960); Canada (Weaver, 2001)
Oomycetes	
Basidiophora entospora Roze & Cornu	Australia (Cunnington and Constantinescu, 2006), Austria (Riethmuller et al., 2002); Bulgaria (Negrean et al., 2004); China(Yu, 1998); Czech Republic (Muller & Kokes, 2008); England, France, Germany, Hungary,Iraq, New Zealand and Romania (Barreto & Dick, 1991); Poland (Mulenko et al., 2008); Portugal (Garcia-Blazquez et al., 2006); USA (Anonymous, 1960)

*Adapted from Farr *et al.*, 2011.

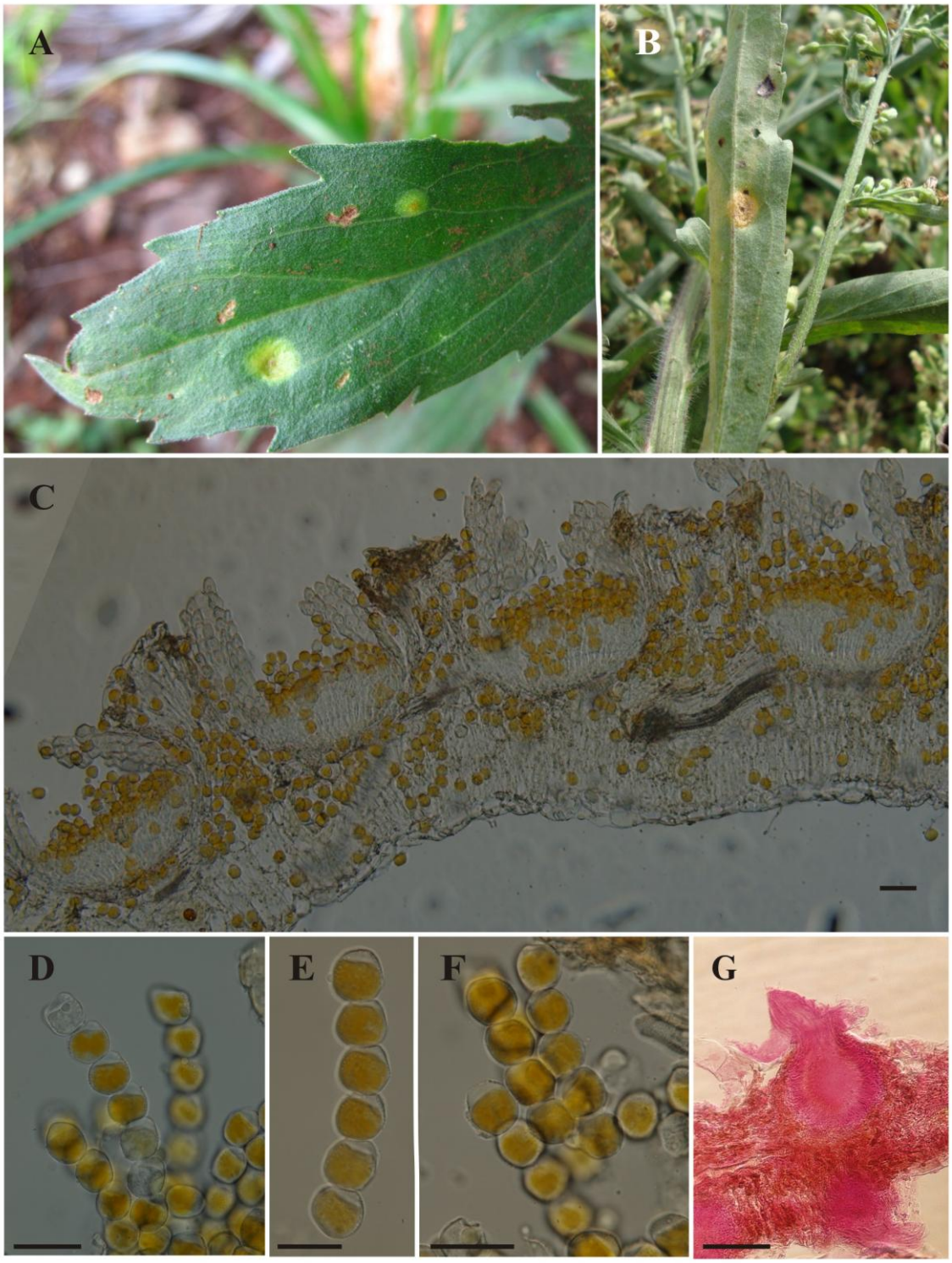

Fig 1. *Aecidium* sp. nov. on *Conyza canadensis*. **A**-**B**. Adaxial and abaxial side of leaf, respectively, showing foliar deformations and yellowing of infected tissues. **C**. Aecia. **D**-**F**. Chains of aeciospores. **G**.Spermogonium. Bars = 30µm.

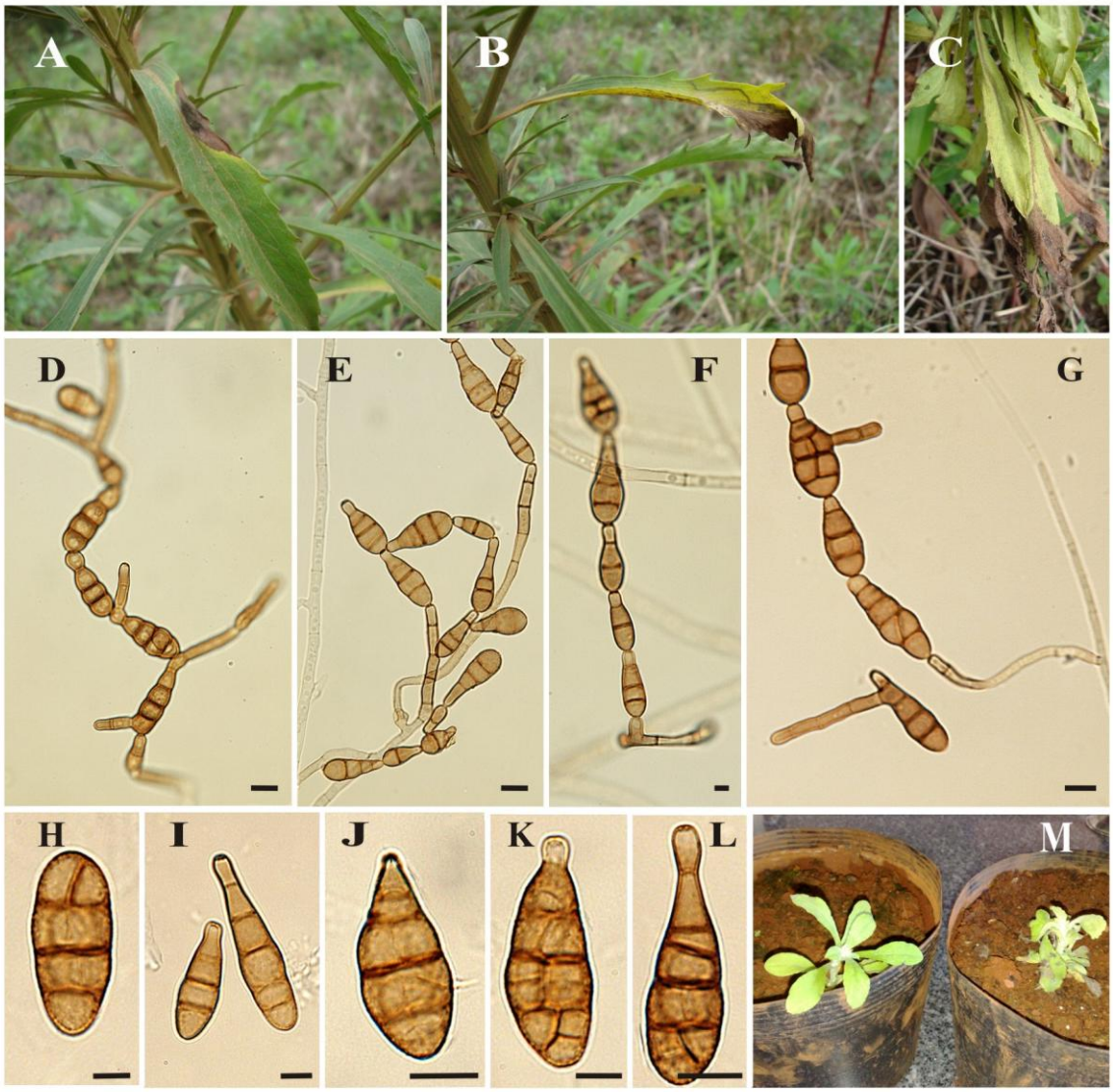

Fig 2. *Alternaria tenuissima* on *Conyza canadensis*. **A**-**C**. Symptons: leaf necrosis. **D**- **G**. Chains of conidia still attached to the conidiophores. **H**. Oval conidium without beak. **I**. Obclavate conidia. **J**-**K**. Oval conidia. **L**. Obclavate muriform conidium. **M**. Pathogenicity test, at left control plant. Barrs = 10µm.

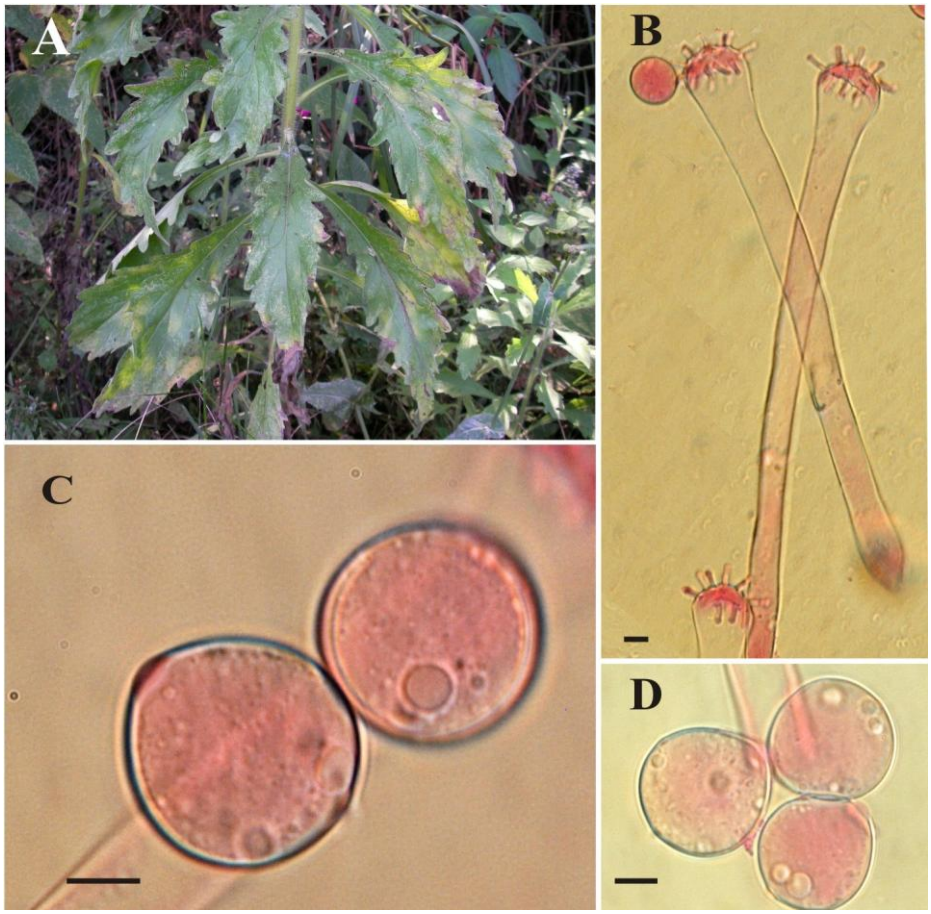

Fig 3. *Basidiophora entospora* on *Conyza canadensis*. **A**. Symptons: leaf showing yellowing. **B**. Sporangiophore. **C**-**D**. Sporangiospores. Barrs = 10µm

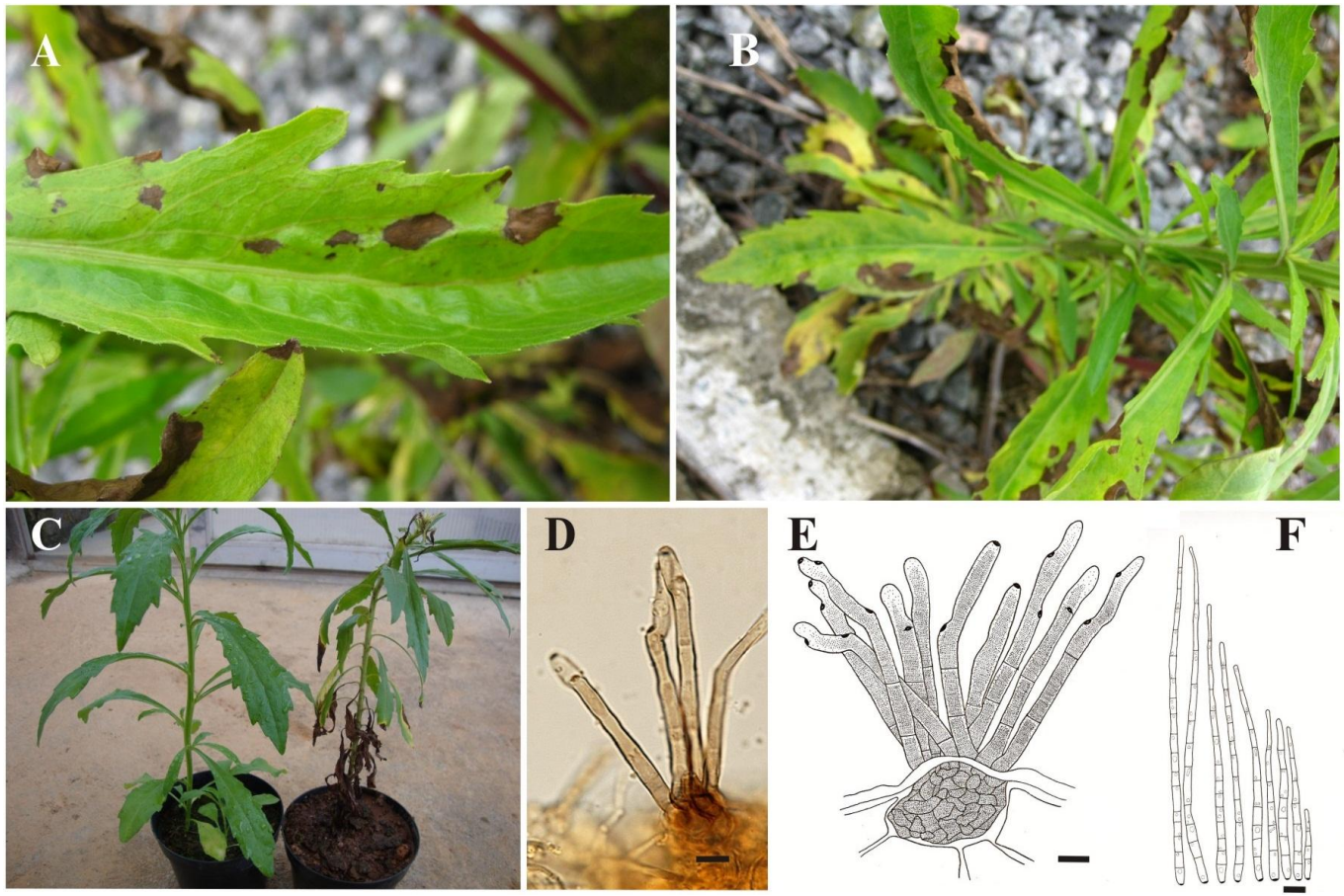

Fig. 4. *Cercospora* sp. nov. on *Conyza canadensis*. **A**-**B**. Symptons: dark brown leaf spots. **C**. Pathogenicity test: control plant (left) inocultated plant (right). **D**. Conidiophores arising through stomata. **E**. Conidiophores and the substomatal stroma. **F**. Filiform conidia. Barrs = 10µm.

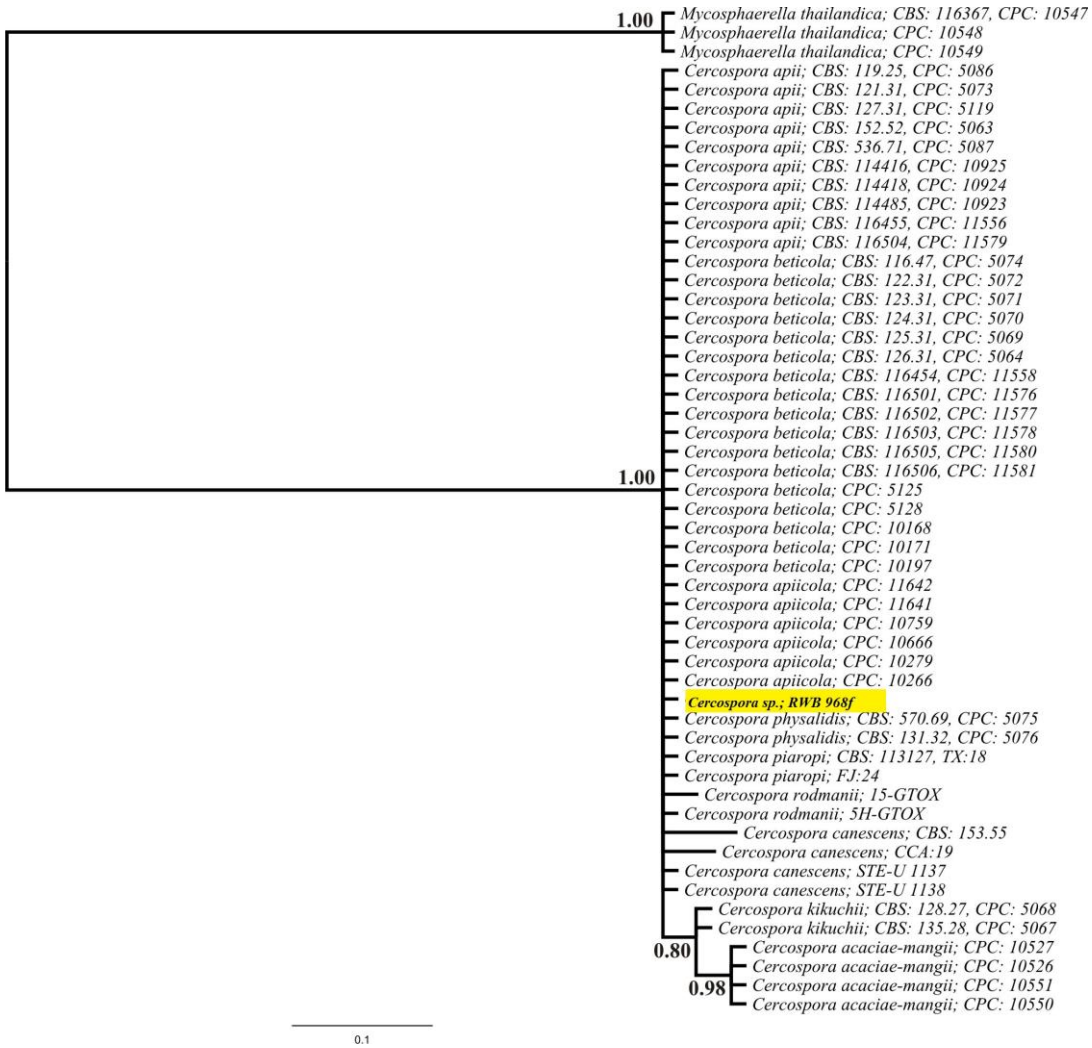

FIG 5. Phylogenetic relationships of *Cercospora* strains inferred by Bayesian analysis of ITS region. The number above the lines represent Bayesian posterior probability values. Highlighted in yellow the *Cercospora* sp. isolate from *Conyza canadensis*.

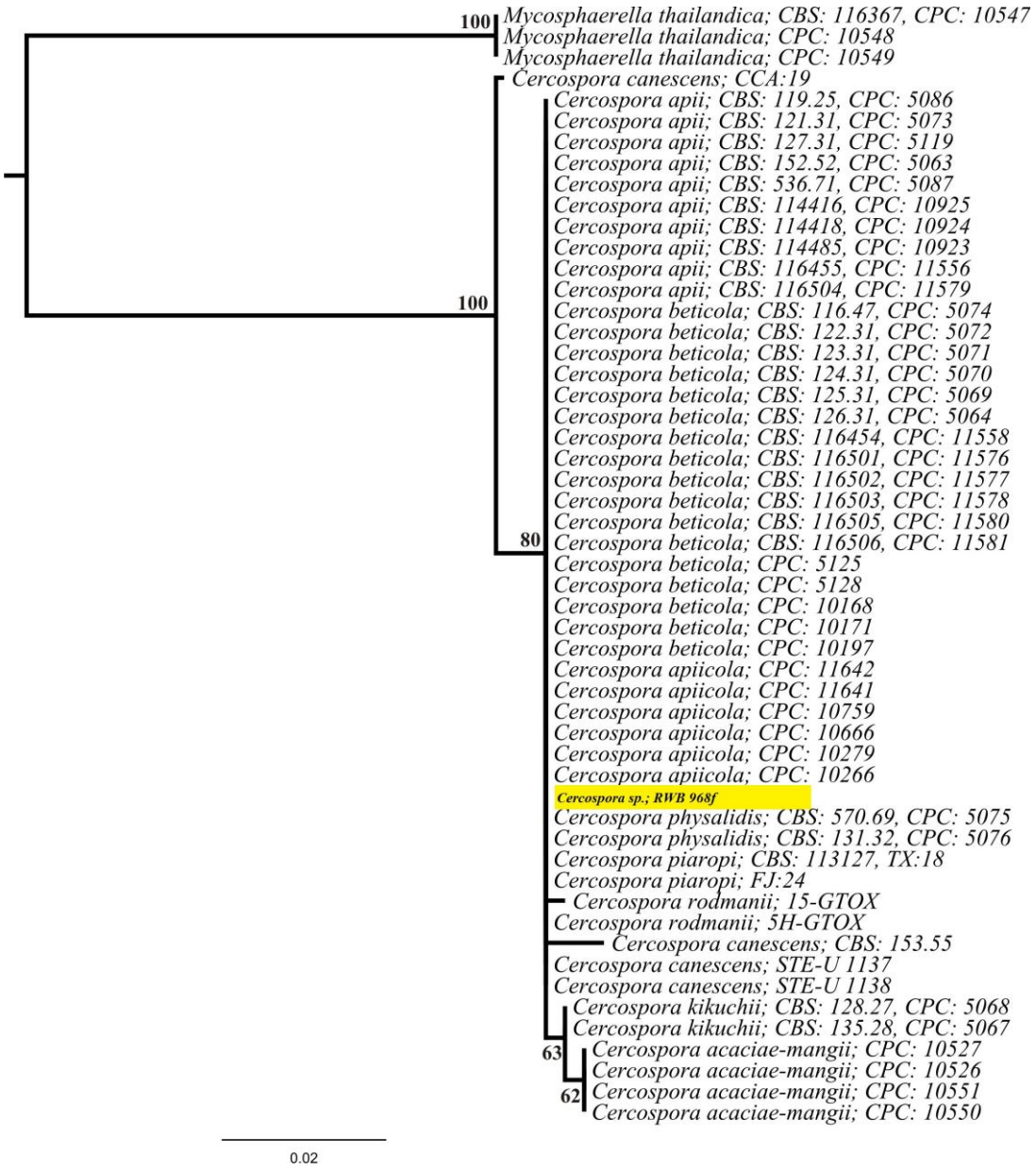

FIG 6. Phylogenetic relationships of *Cercospora* strains inferred by Maximum Likelihood analysis of ITS region. The number above the lines represent the bootstrap (bootstrap=1000) values. Highlighted in yellow the *Cercospora* sp. isolate from *Conyza canadensis*.

FIG 7. Phylogenetic relationships of *Cercospora* strains inferred by Bayesian analysis of part of calmodulin gene. The number above the lines represent Bayesian posterior probability values. Highlighted in yellow the *Cercospora* sp. isolate from *Conyza canadensis*.

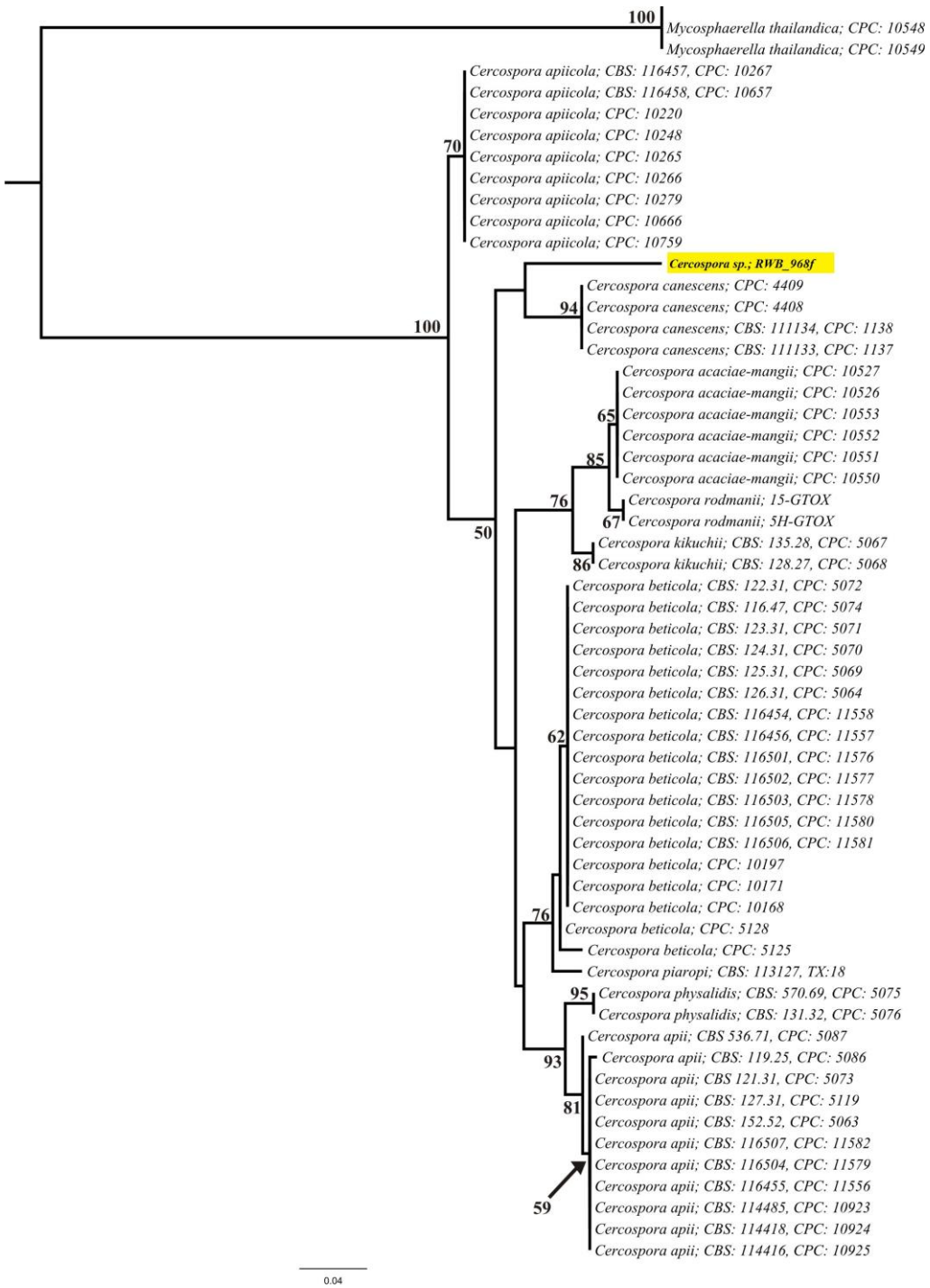

FIG 8. Phylogenetic relationships of *Cercospora* strains inferred by Maximum Likelihood analysis of part of calmodulin gene. The number above the lines represent the bootstrap (bootstrap=1000) values. Highlighted in yellow the *Cercospora* sp. isolate from *Conyza canadensis*.

FIG 9. Phylogenetic relationships of *Cercospora* strains inferred by Bayesian analysis of part of translation elongation factor gene. The number above the lines represent Bayesian posterior probability values. Highlighted in yellow the *Cercospora* sp. isolate from *Conyza canadensis*.

FIG 10. Phylogenetic relationships of *Cercospora* strains inferred by Maximum Likelihood analysis of part of translation elongation factor gene. The number above the lines represent the bootstrap (bootstrap=1000) values. Highlighted in yellow the *Cercospora* sp. isolate from *Conyza canadensis*.

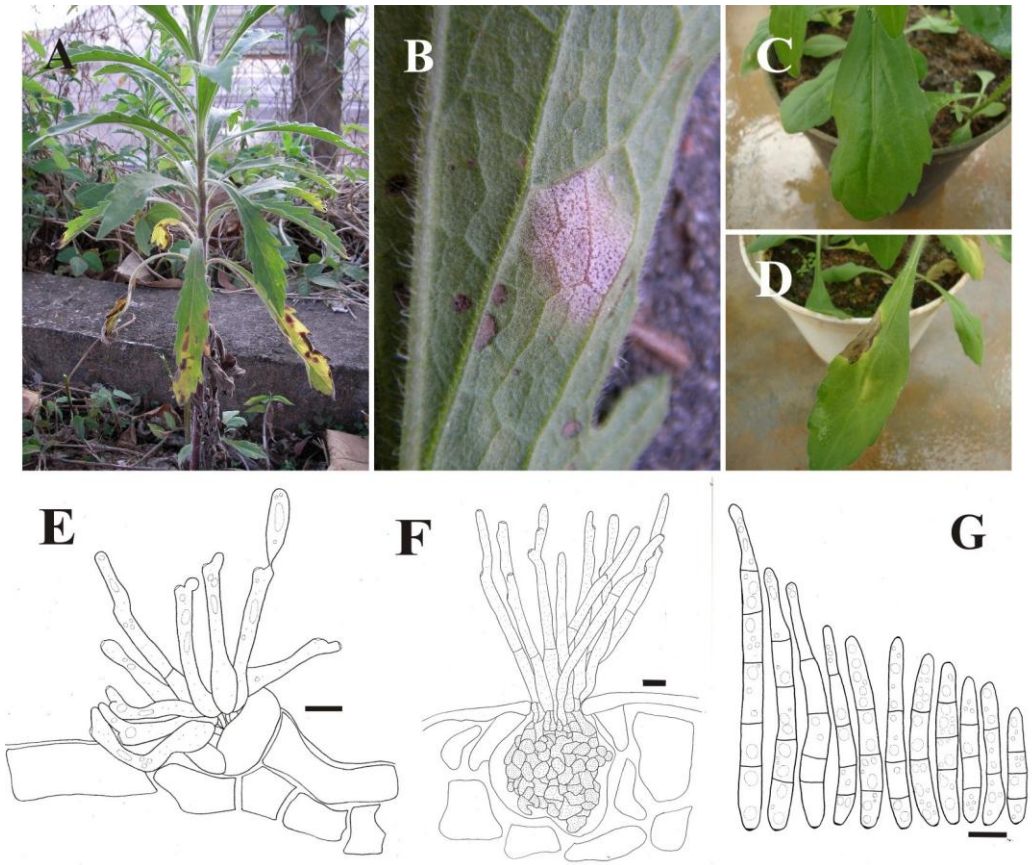

Fig 11. *Cercosporella virgaureae* on *Conyza canadensis*. **A**. Leaves showing necrosis and a yellowing halo.**B**. Intense sporulation abaxially. **C**-**D**. Pathogenicity test: Control plant (**C**) and inoculated plant (**D**). **E**. Conidiophores arising through stomata. **F**. Conidiophores and the substomatal stroma. **G**. Conidia. Barrs = 10µm.

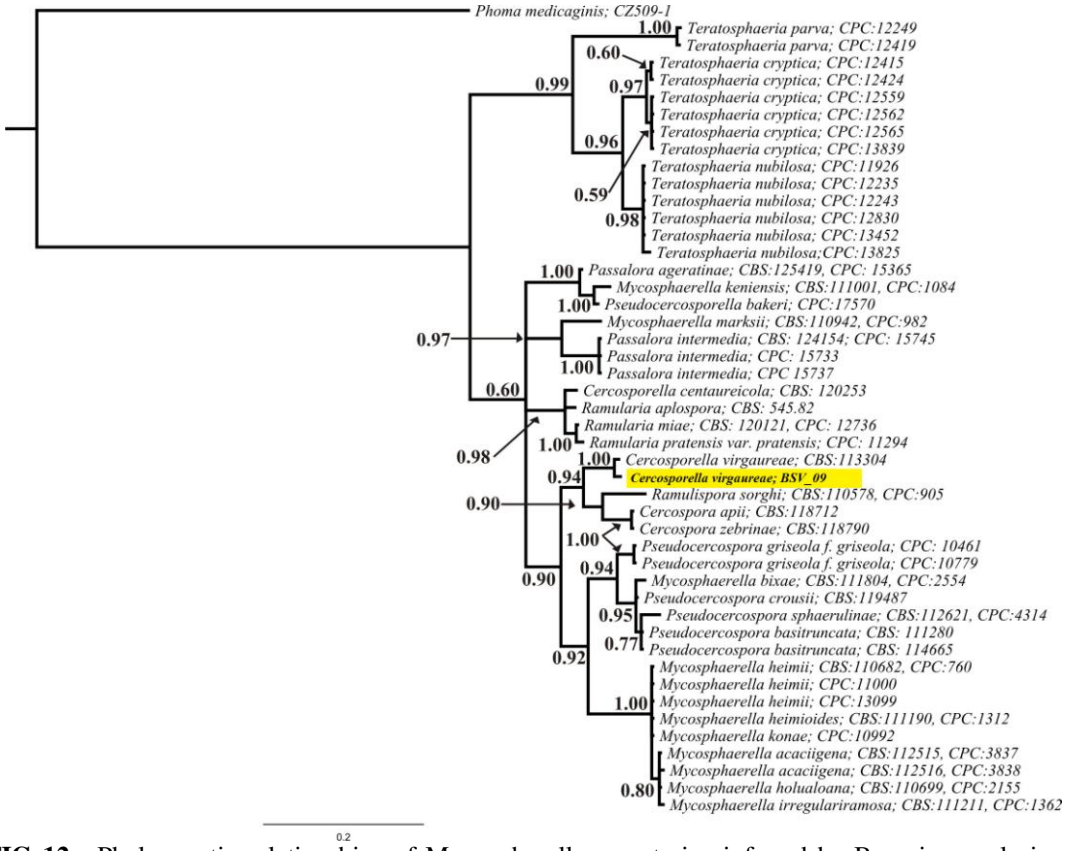

FIG 12. Phylogenetic relationships of Mycospharellaceae strains inferred by Bayesian analysis of part of large subunit ribosomal region. The number above the lines represent Bayesian posterior probability values. Highlighted in yellow the *Cercosporella virgaureae* isolate from *Conyza canadensis*

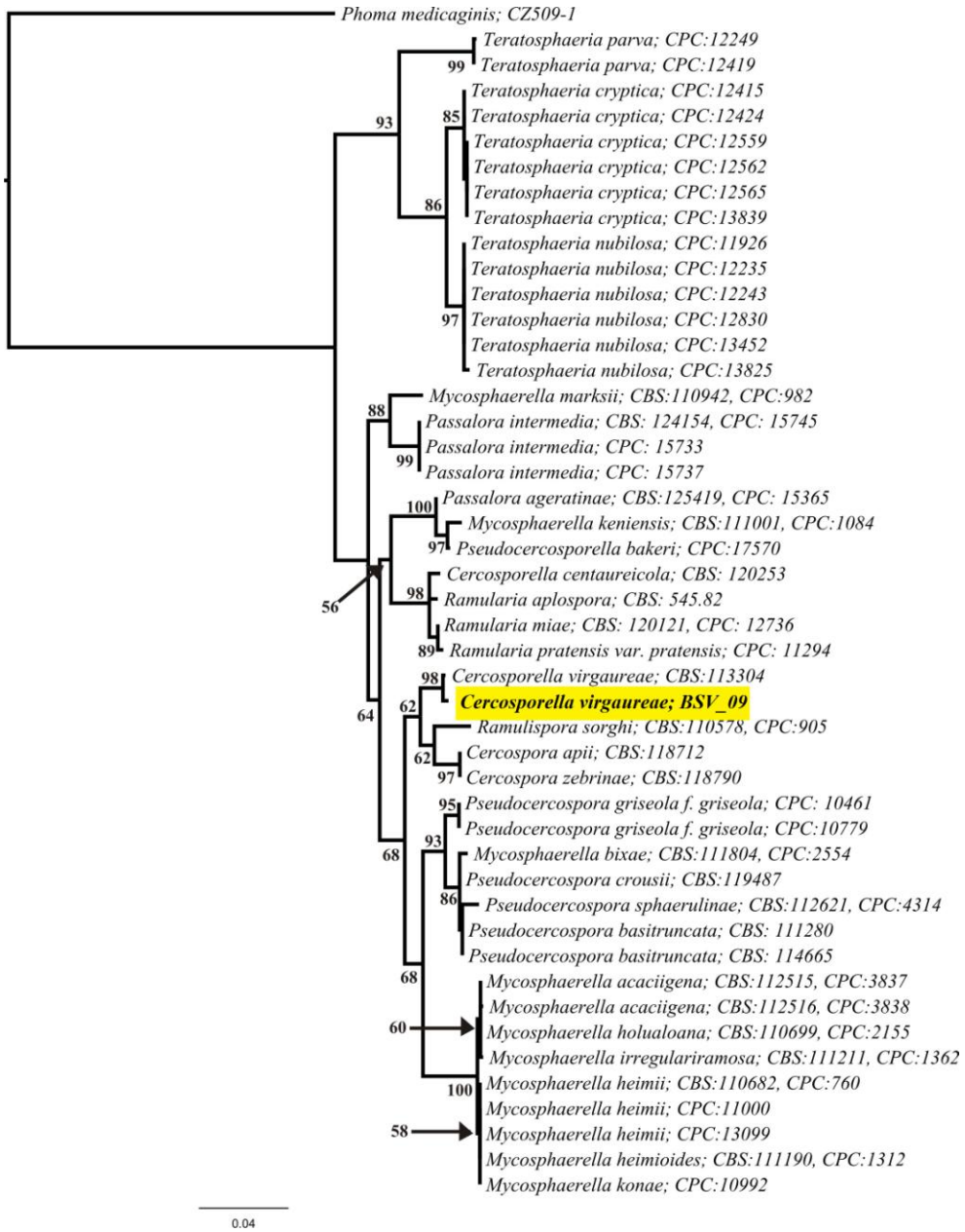

FIG 13. Phylogenetic relationships of Mycospharellaceae strains inferred by Maximum Likelihood analysis of part of large subunit ribosomal region. The number above the lines represent the bootstrap (bootstrap=1000) values. Highlighted in yellow the *Cercosporella virgaureae* isolate from *Conyza canadensis*.

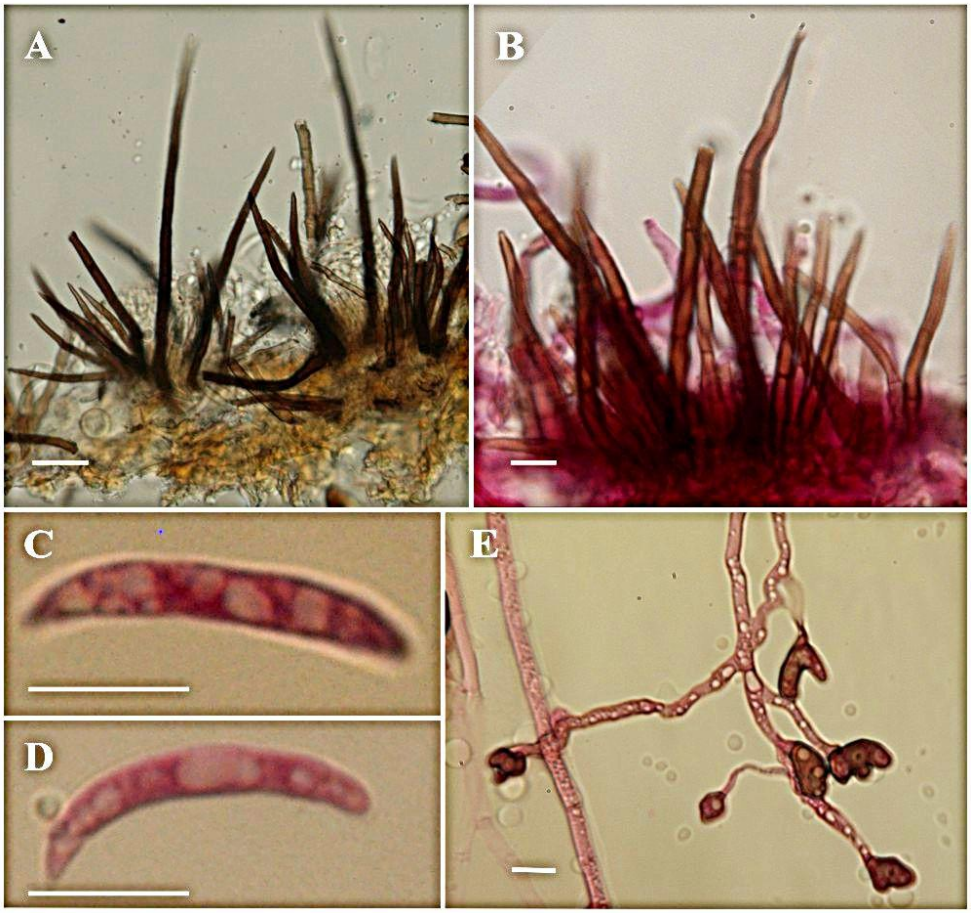

Fig 14. *Colletotrichum capsici* on *Conyza canadensis*. **A**-**B**. Acervuli with setae. **C**-**D**. Falcate conidia. **E**. Lobated appressoria. Barrs = 10µm.

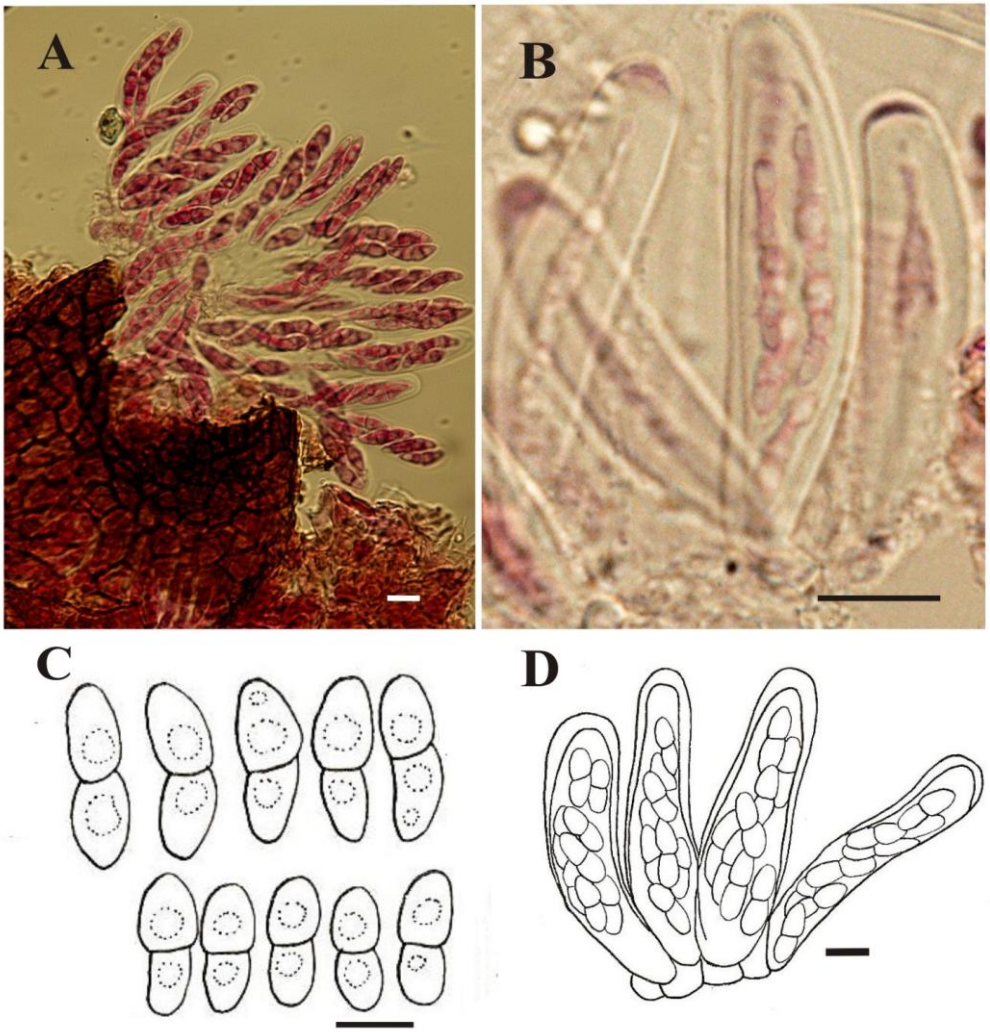

Fig 15. *Mycosphaerella* sp. on *Conyza canadensis*. **A**. Pseudothecia and asci. **B**. Bitunicate asci with young ascospores. **C**. Ascospores (note the stronge strangulation at the septum). **D**. Asci and ascospores. Barrs = 10µm.

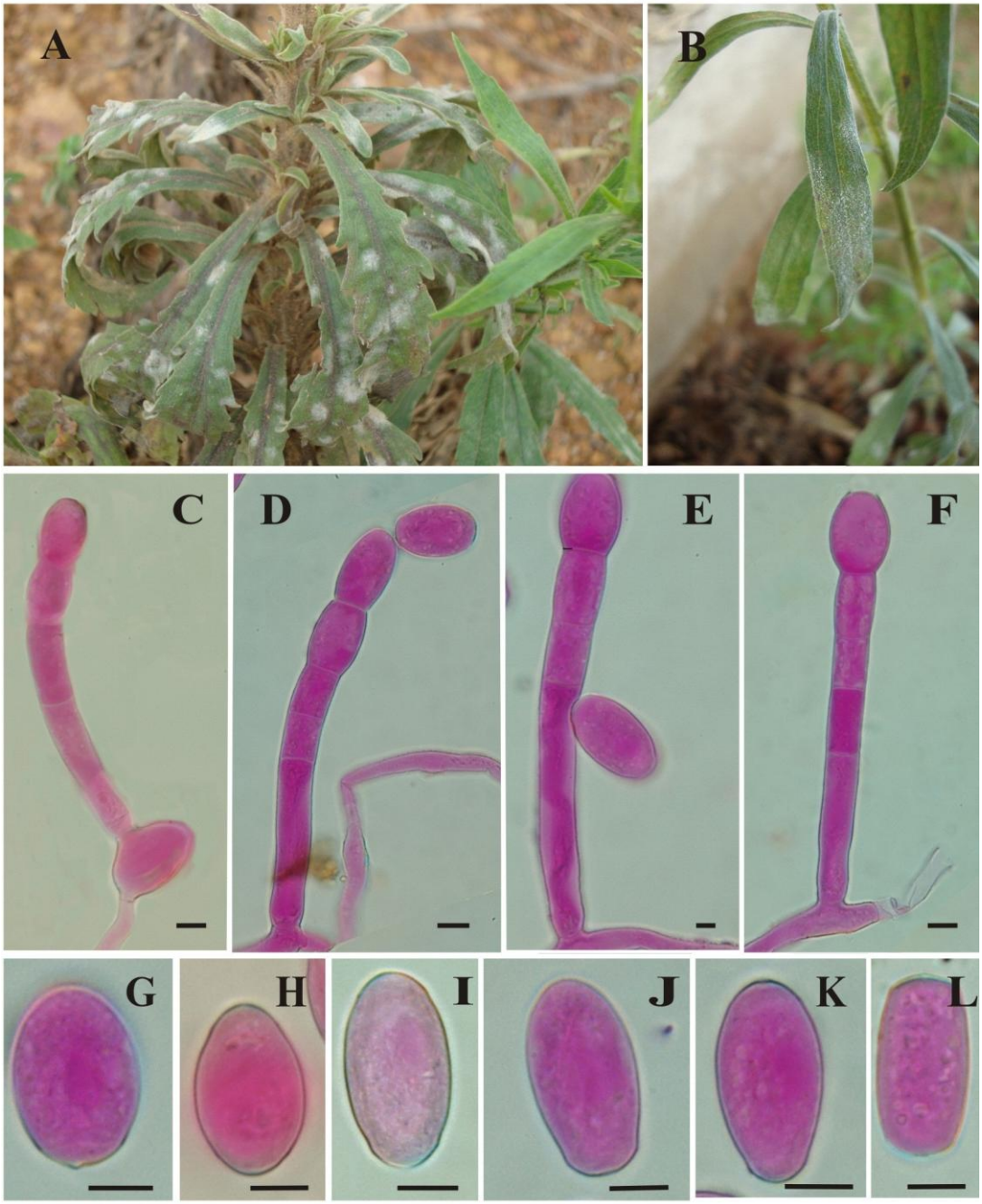

Fig 16. *Podosphaera fusca* on *Conyza canadensis*. **A**. **B**. Leaves showing powdery mildew infection. **C**. Conidiophores arising directly from conidia. **D-F**. Conidiophores and conidia. **G- L**. Range of conidial shape.**G-H**. Ovoid. **I**. Doliform **J-K**. Ellipsoid. **L**.Cylindric. Barrs = 10µm.

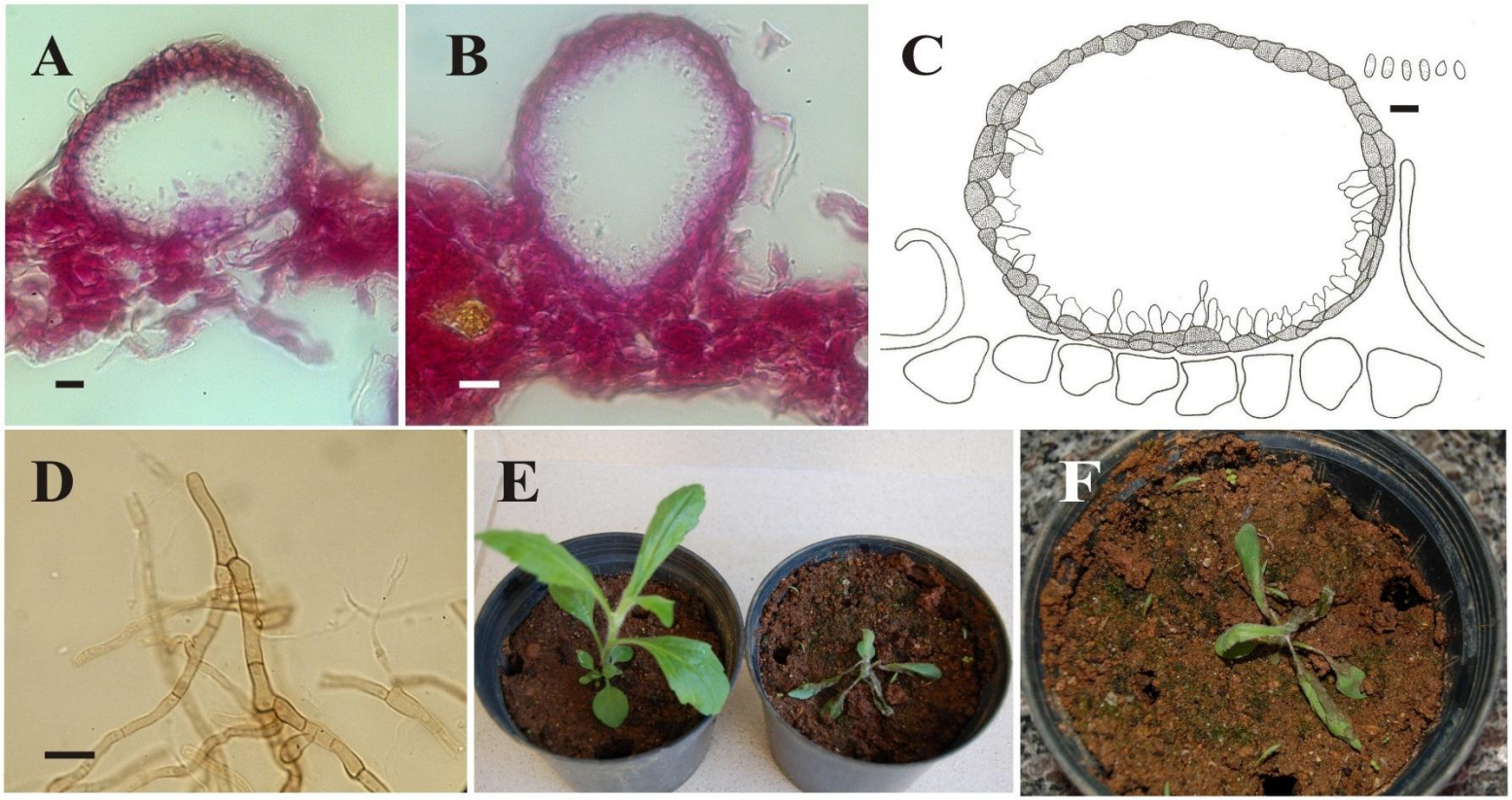

Fig 17. *Phoma canadensis* on *Conyza canadensis*. **A**, **B**. Pycnidia. **C**. Pycnidia with conidiogenous cells and conidia at the right top. **D**. Mycelium produced in MEA medium. **E**. Pathogenicity test: Control plant (left) inoculated plant (right). **F**. Closer look at the inoculated plant. Barrs = 10 µm.

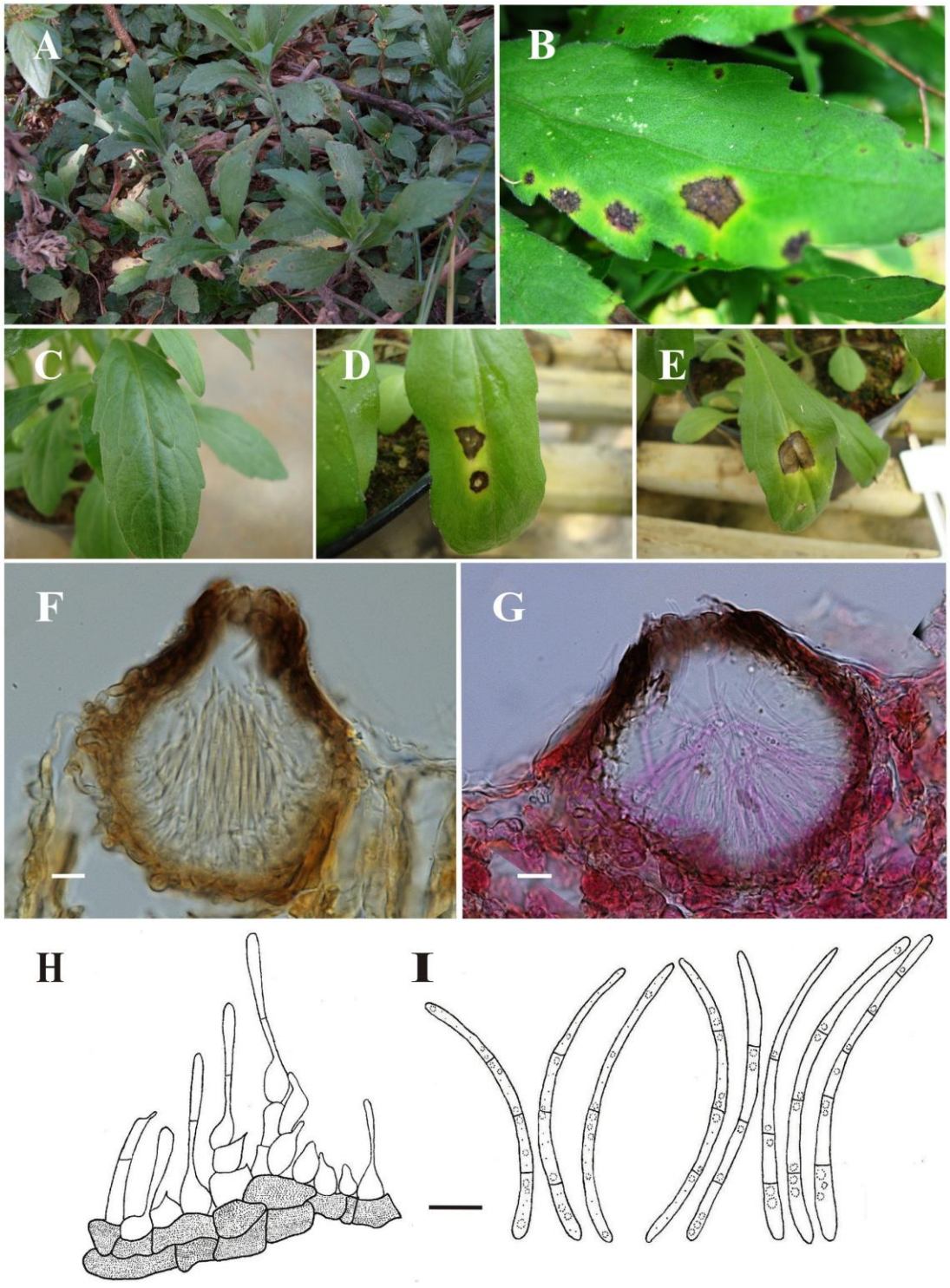

Fig 18. *Septoria erigerontis* on *Conyza canadensis*. **A**, **B**. Symptons: leaves with necrosis (greyish at center) surrounded by a yellowing halo. **C**-**E**. Pathogenicity test. **C**. Control plant. **D**, **E**. Inoculated plants. **F**-**G**. Pycnidia. **H**. Conidiogenous cells. **I**. Filiform conidia. Barrs = 10µm.

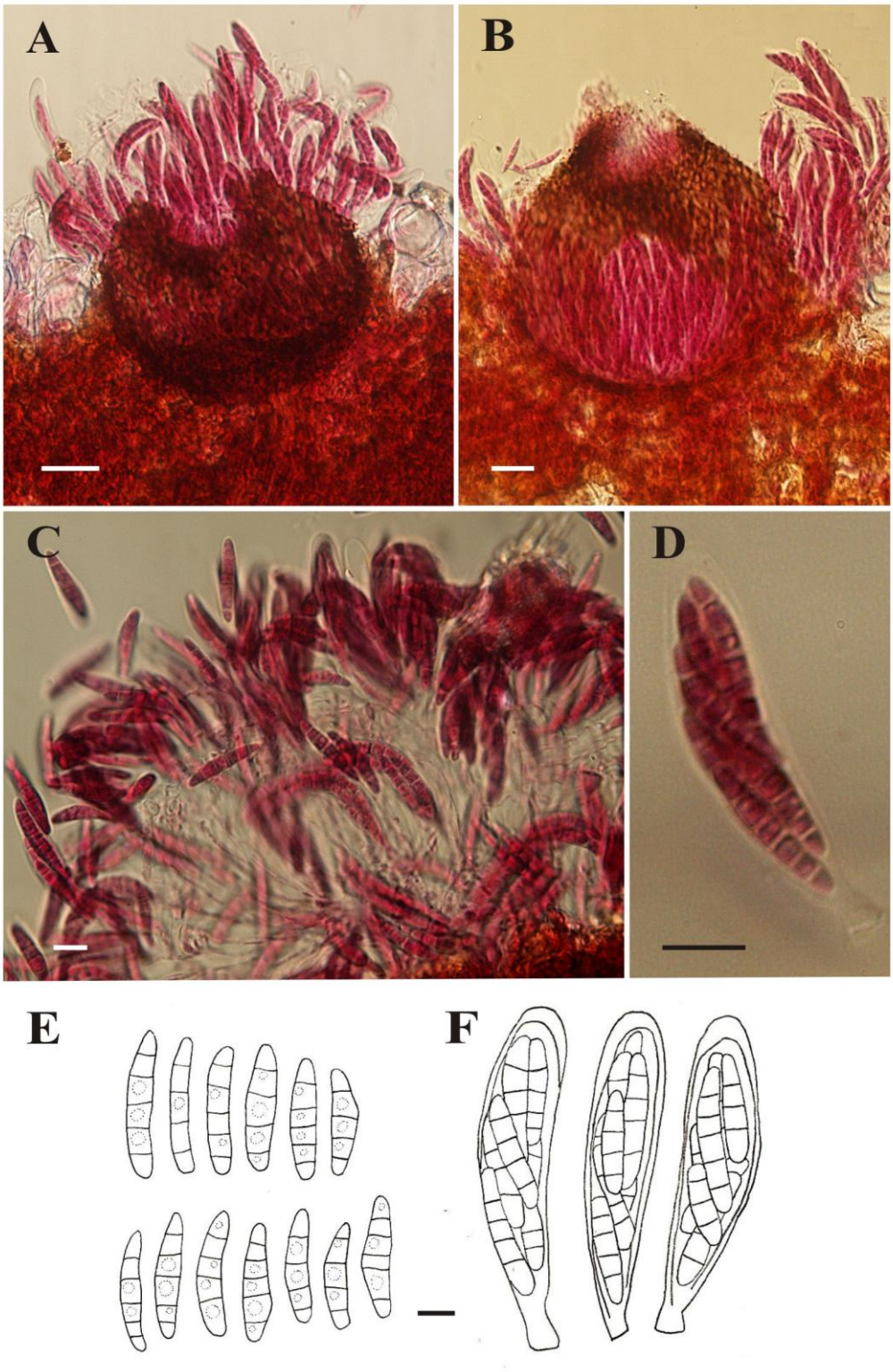

Fig 19. *Sphaerulina* sp. nov. on *Conyza canadensis*. **A**, **B**.Pseudothecia with asci. **C**. Numerous asci with ascospores. **D**. Closer look at the asci. E. Ascospores. **F**.pedicellate asci with ascospores. Barrs = 10µm.

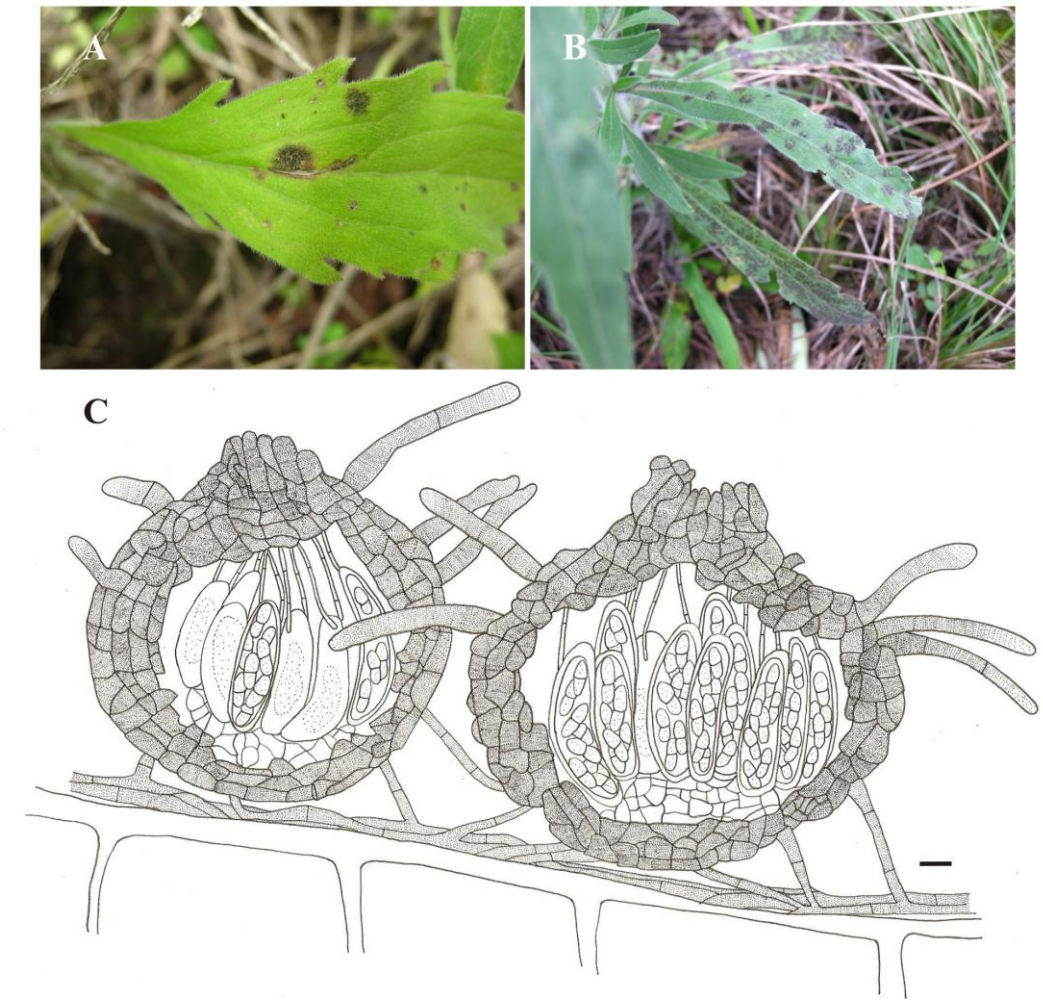

Fig 20. *Wentiomyces melioloides* on *Conyza canadensis.* **A**, **B**. Black colonies under the leaves. **C**. Pseudothecia with asci, ascospores and the interthecial filaments. Barrs = 10µm.