Biological control of *Tradescantia fluminensis* with pathogens
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Introduction

Tradescantia fluminensis (wandering Jew; local name in Brazil – trapoeraba) is one among a series of weed species of world importance belonging to the Commelinaceae. It is native to South America, and is particularly common along the coast in Southeastern and Southern Brazil where it forms small patches on humid rocky habitats such as along creek margins. It never forms dense extensive populations and it is not regarded as a weed of importance in Brazil. Conversely in situations where it was introduced into exotic tropical and subtropical regions of the world it became a very serious invader of native ecosystems. It is particularly harmful to forest ecosystems in New Zealand, affecting invertebrate communities (Toft et al., 2001; Standish, 2004), hampering natural processes of forest regeneration and nutrient cycling (Standish et al., 2001; Standish, 2002, Standish et al., 2004). It has no significant natural enemies (arthropods or pathogens) in New Zealand (Winks et al., 2003). Although Brazil is considered to be the center of origin of *T. fluminensis*, until this work was started there was not a single pathogen recorded to be associated with this plant species (Table 1).

Table 1 Fungal pathogens recorded on *Tradescantia fluminensis* (Petrak, 1950; Gómez and Kisimova-Horovitz, 1997; Waipara, 2006; Farr et al., 2010).

Fungal species	Distribution
Alternaria sp.	USA
Botrytis cinerea	USA
Cercospora sp.	USA
Cladochytrium replicatum	USA
Kordyana tradescantiae	Ecuador, Costa Rica
Phakopsora tecta	Argentina
Pythium sp.	Hawaii
Rhizoctonia sp.	USA
Sclerotinia sclerotiorum	New Zealand
Uromyces commelinae	Uruguay

The cooperative research project initiated between the Universidade Federal de Viçosa (Brazil) and Manaaki Whenua Landcare Research New Zealand Ltd. aimed at surveying and evaluating the native pathobiota associated with *T. fluminensis* in Brazil for potential classical biological control agents led to the discovery of a number of pathogens, some of which are being described as new records for Brazil, new host-records and taxonomic novelties separately. Part of these results were published recently (Pereira et al., 2008). Here an overview of what has been achieved so far on the studies aimed at the use of plant pathogens as biocontrol agents of *T. fluminensis* is presented.

Surveys

The field survey was systematic and involved all states of Southern Brazil. Records of *T. fluminensis* were compiled from eight Brazilian herbaria. The following Southern and Southeastern Brazilian states were visited during January 2003, November-December 2005, July 2008, November 2009: Minas Gerais, Rio de Janeiro, Espírito Santo, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul. Further details of the procedure adopted for the systematic survey can be found in Barreto and Evans (1994). The diseased parts of the plants suspected to be damaged by fungal or bacterial pathogens were collected, dried in a plant press and taken to the laboratory. Seedlings infected by biotrophic fungi were also brought to the laboratory in Viçosa (MG). Fungal structures were removed from specimens and mounted in lactophenol or lactofucsin. Observations of morphology, measurements and illustrations were carried out with an OLYMPUS BX 50 light microscope fitted with a drawing tube. Isolations were conducted by collecting conidia or ascospores from sporulating lesions with a fine pointed needle and plating them on Vegetal Broth Agar medium (Pereira et al., 2003). The isolates of non-biotrophic fungi were stored on silica gel according to Dhingra and Sinclair (1995). The materials examined were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC). Additional materials previously deposited at VIC were also examined.

Nine fungal species regarded as potentially pathogenic to *T. fluminensis* were collected on *T. fluminensis* or closely related taxa along five years of survey. Three of these were found to be new to science and their description is being published separately. The fungal taxa that were collected and the diseases they cause are as follows: *Ceratobasidium tradescantiae* (mild blight) (Fig 1); *Cercospora apii* (leaf spot) (Fig 2); *Colletotrichum falcatum* (anthracnose) (Fig 3); *Kordyana brasiliensis* sp nov. (white smut) (Fig 4,5); *Mycosphaerella tradescantiae* (leaf spot) (Fig 6-7); *Rhizoctonia solani* (blight) (Fig 8); *Sclerotium rolfsii* (collar rot) (Fig 9); *Septoria paranaensis* sp. nov (leaf spot) (Fig 10) e *Uromyces commelinae* (rust) (Fig 11).
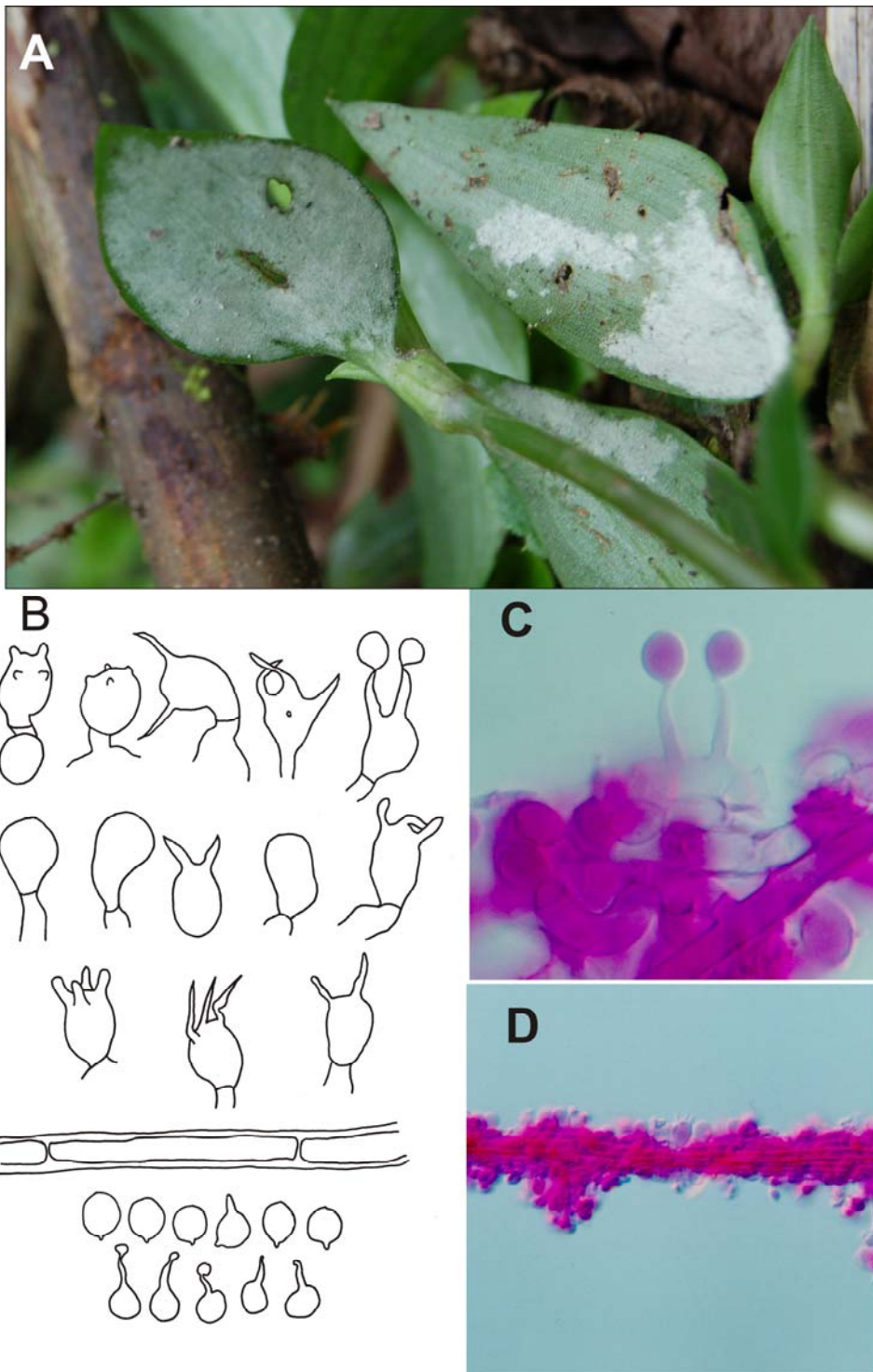
Fig 1.A. Mild blight symptoms caused by *Ceratobasidium tradescantiae* on *T. fluminensis.* **B-C.** Basidia and basidiospores of *C. tradescantiae.* **D**. Mycelial rope with basidia and basidiospores.
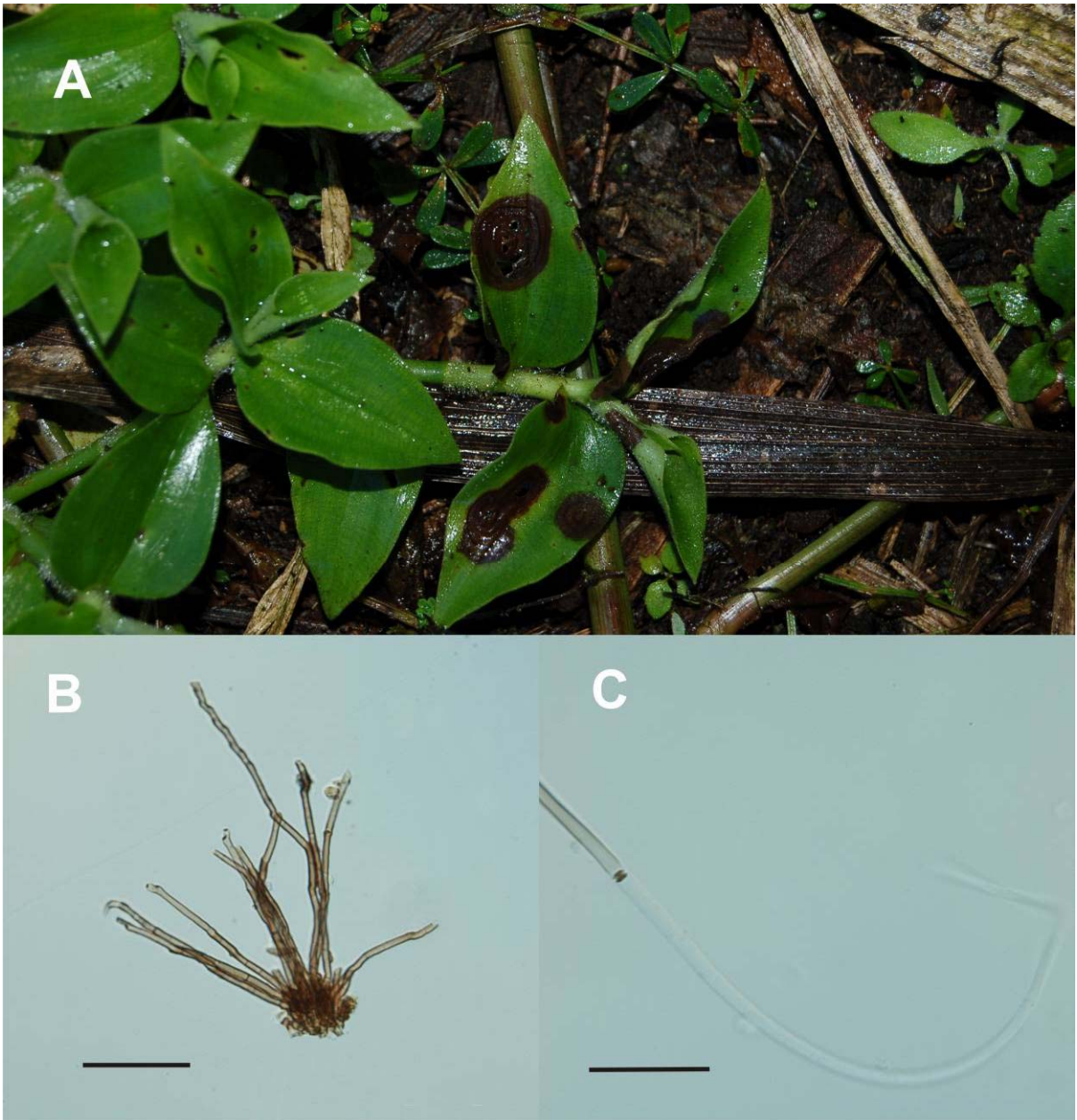
Fig 2. A. Leaf spots on *T. fluminensis* caused by *Cercospora apii* **B.** Conidiophores of *C. apii* (Bar = 60 μm) **C.** Conidium of *C. apii* still attached to a conidiogenous cell (Bar = 30 μm)
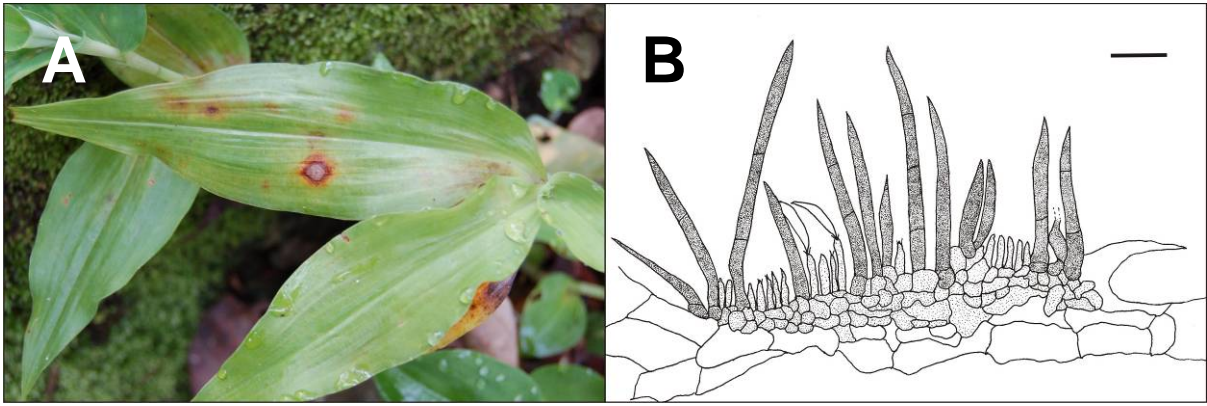
Fig 3. *Colletotrichum falcatum* : **A.** Anthracnose symptoms on *T. fluminensis* leaves.

B. Acervulus. (Bar=20.0 μm).

Fig 4 Population of *Tradescantia fluminensis* bearing white smut symptoms caused by *Kordyana brasiliensis*: **A.** General view. **B-** Close-up (note intense yellowing of lower leaves with brown necrotic tissues appearing centrally).
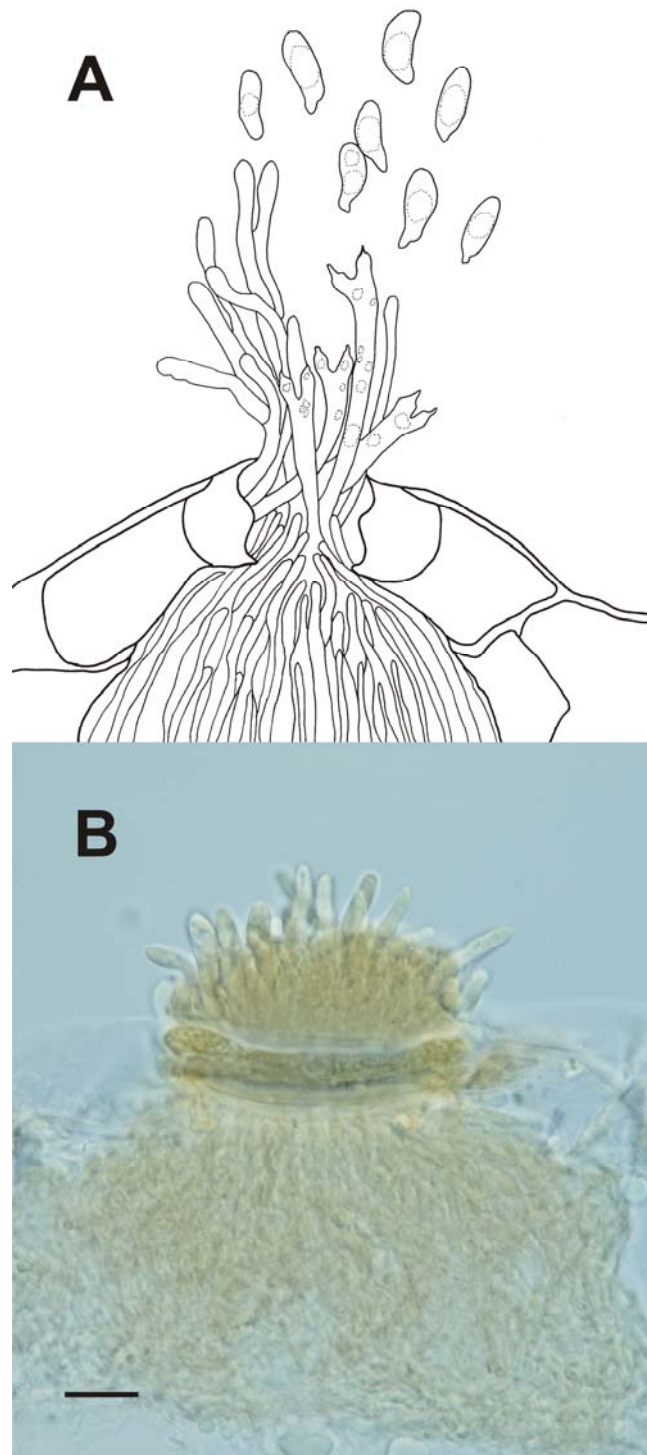
Fig 5 A - Caespituli of basidia of *Kordyana brasiliensis* emerging through stoma of *Tradescantia fluminensis.* **B** - *Ibid* (Bar= 15 µm).

Fig 6.A. Leaf spots on *T. fluminensis* caused by *Mycosphaerella tradescantiae* adaxially (right) and abaxially (left*).*
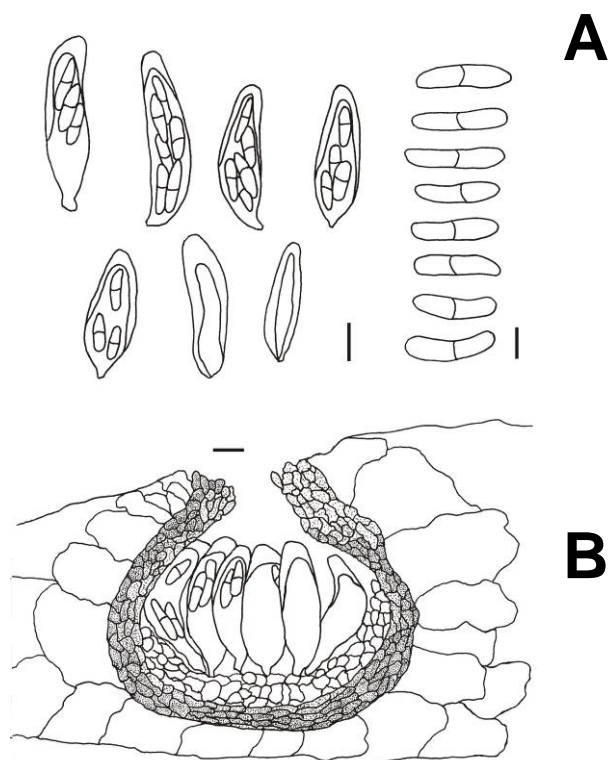

Fig 7. *Mycosphaerella tradescantiae*:   **A**. Asci and ascospores (Bar=10.0 µm) **B** Section through a pseudothecium.
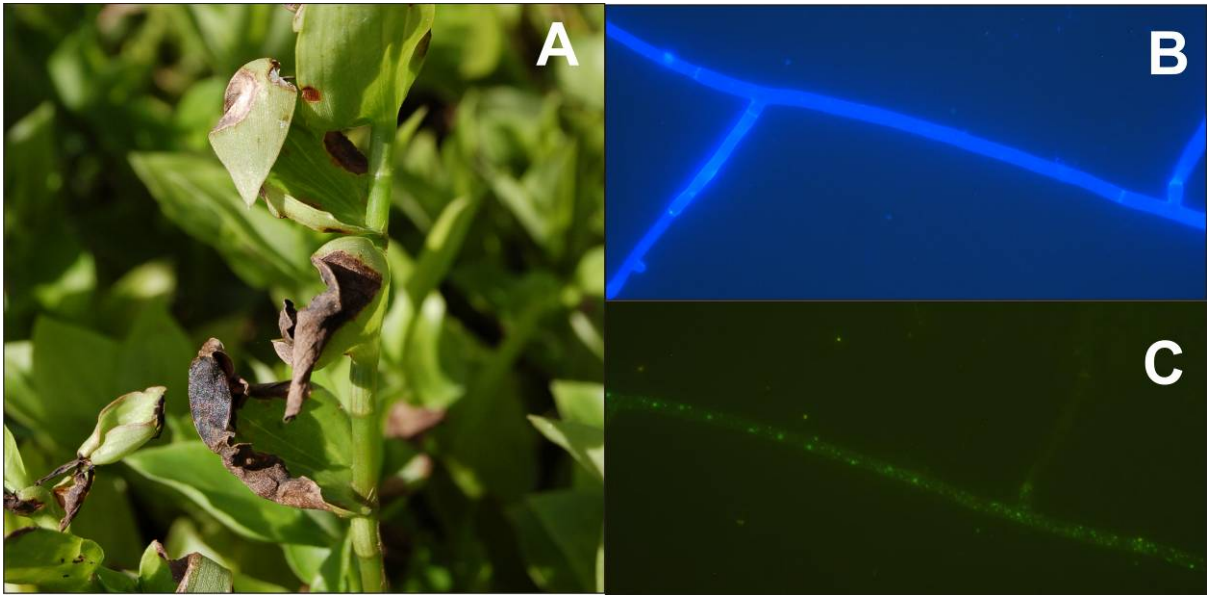
Fig 8. *Rhizoctonia solani*: **A.** Symptoms of blight on *T. fluminensis*. **B-C.** Mycelium stained with SybrGreen® showing the multinucleate condition of cells.

Fig 9. *Sclerotium rolfsii*: **A.** *T. fluminensis* plants killed by the fungus (Note abundant sclerotia formed on dead leaves and stems). **B.** Abundant production of sclerotia of *S. rolfsii in vitro.*

Fig 10. *Septoria paranaensis* **A.** Leaf spots on *T.* (viz.) *fluminensis* . **B.** Pycnidium, conidiogenous cells and conidia (Bar= 45.0 μm).

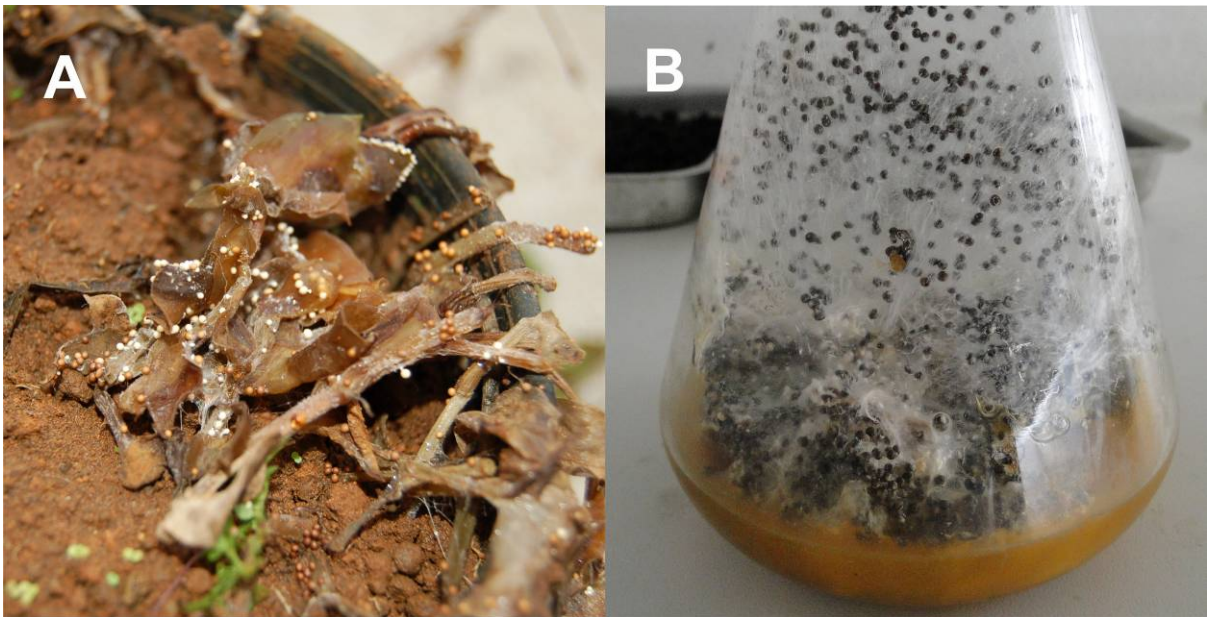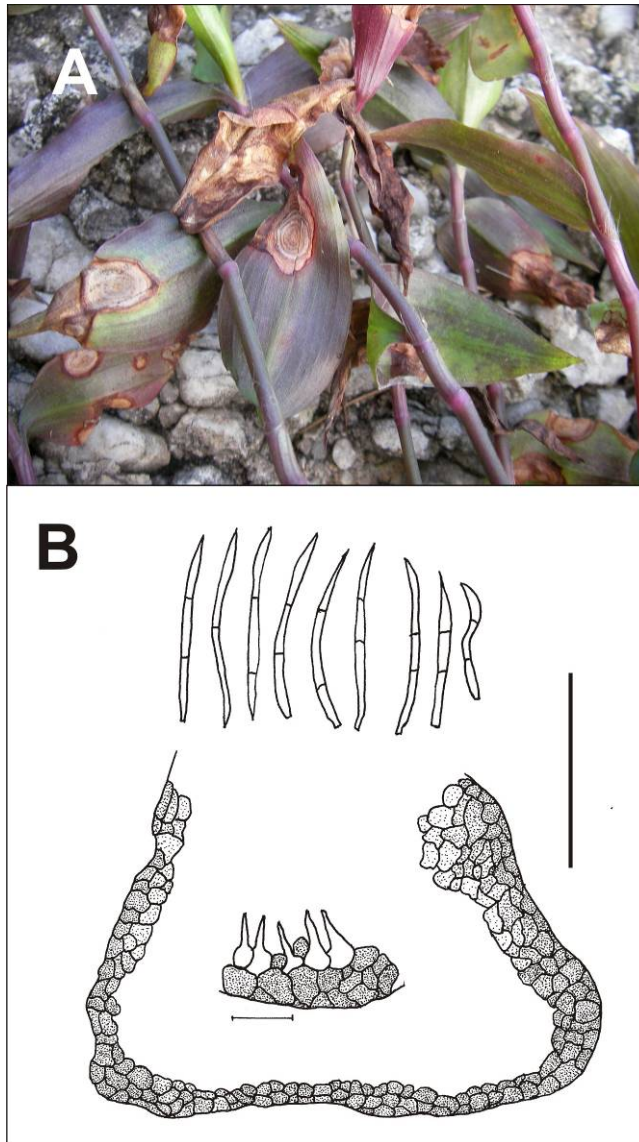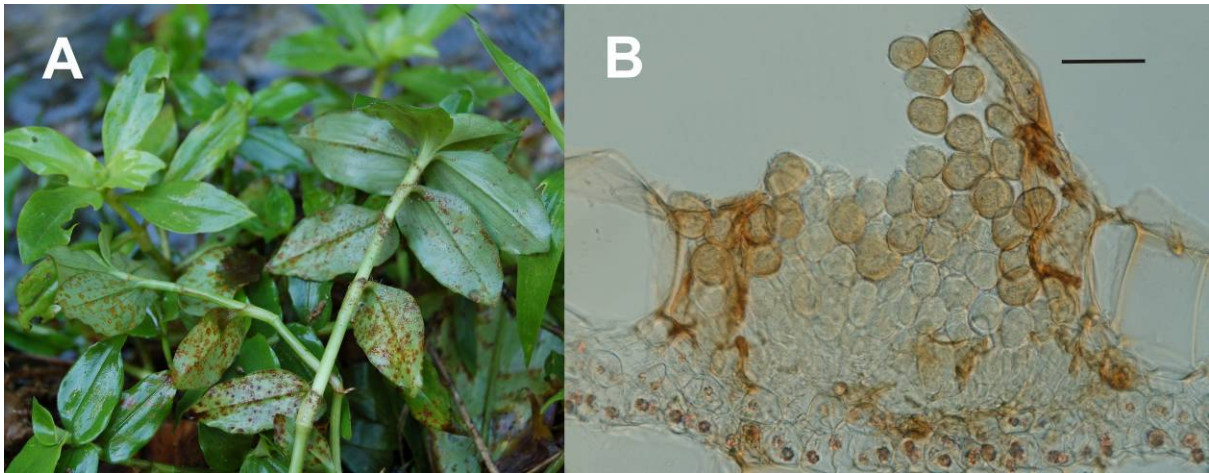**Fig 11.** *Uromyces commelinae* **A.** Uredinia formed adaxially on infected leaves of *T. fluminensis*. **B.** Uredinia and urediniospores of *U. commelinae.* (Bar=25.0 µm).

Information on recorded distribution of the fungal pathogens collected on *T. fluminensis* is given below in Table 2.

Table 2. Fungal pathogens collected on *Tradescantia fluminensis* or closely related species during field surveys in Brazil

Fungal species	Distribution in Brazilian states*
Ceratobasidium tradescantiae	SC (2); RS (2)
Cercospora apii	MG (2); PR (2); SC (1); SP(1)
Colletotrichum falcatum	RS(1)
Kordyana brasiliensis	MG (1); PR(4); SC(3); SP(1); RS (9)
Mycosphaerella tradescantiae	RS (1)
Rhizoctonia solani	RJ(1)
Septoria paranaensis	PR(1)
Sclerotium rolfsii	MG(1)
Uromyces commelinae	PR (1); SC (2); RS (5)

* The numbers in parentheses represent how many times each fungus was collected in a state (MG – Minas Gerais; PR – Paraná; RJ - Rio de Janeiro; RS – Rio Grande do Sul; SC – Santa Catarina; SP – São Paulo).

Additionally a phytopathogenic bacterium was collected on this host in the state of Rio de Janeiro and later also in the state of Paraná causing leaf spots that evolved to blight of the plants both on *Tripogandra diuretica* (Commelinaceae) and *T. fluminensis*. This bacterium was recognized as a severe pathogen capable of developing systemic infections on its host as observed on infected plants brought back to Viçosa. It was identified as *Burkholderia andropogonis* (Smith, 1911) through a combination of classical evaluation of features such as colony morphology and physiology and sequencing of 16S rRNA and analysis of sequence data. A draft publication including information on host-range was prepared on this topic but this was based on a single isolate, which led to criticism and withdrawal of the manuscript (still awaiting collection of additional isolates). Unfortunately a combination of practical difficulties combined with the higher priority given to fungal diseases namely the rust and the white smut led to a temporary abandonment of this potential biocontrol agent.

Culturability

Seven of the fungal pathogens were isolated in pure culture: *C. apii*, *C. falcatum*, *K. brasiliensis*, *M. tradescantiae*, *R. solani*, *S. rolfsii and S. paranaensis.* Repeated attempts to isolate *C. tradescantiae* associated with mild blight were unsuccessful. We believe that this fungus is in fact a biotroph, since, besides its seemingly inability to grow in culture it was observed that even a complete colonization of the abaxial surface of leaves (easily observed by an extensive external coverage of the tissues by a mycelial mat) by *C. tradescantiae* was often unaccompanied of any sign of necrosis. Attempts to isolate *Kordyana* sp. directly from infected leaves onto Vegetable-Broth-Agar (VBA) were firstly unsuccessful. Several other culture media were also tried such as Potato-Dextrose-Agar (PDA), Corn-Meal-Agar (CMA), Potato-Carrot-Agar (PCA) (Dhingra and Sinclair, 1995) and MNM (Melin-Norkrans Modified Medium) (Marx, 1969). Although there is little published information on *Kordyana*, which is a rather obscure fungal genus, this is known to have connections with other basidiomycetes which are known to grow in culture forming yeast-like colonies. Yeast colonies were formed on MNM but filamentous colonies also appeared during isolations and were also kept for further studies.

Elucidation of the identity of the white smut fungus

Although morphology indicated that the fungus on *T. fluminensis* in Brazil was a new species of *Kordyana*, this is a genus of fungi that have a rather simple morphology and for which there is limited published information. Distinction of taxa within this genus thus benefits from the use of molecular information. Additionally, the doubts that emerged about the true identity of the various isolates obtained in culture led us to perform DNA LSU sequence identification of such pure cultures obtained from the white smut infected leaves of *T. fluminensis.* Unfortunately, this showed that none of the isolates belonged to *Kordyana*.

A later round of isolations involving basidiospore ejection onto VBA plates was eventually successful for isolating the white smut fungus as proven by LSU sequence identification.

In the meantime, LSU sequence identification of the white smut as a *Kordyana* species was obtained by extracting DNA directly from the colonized leaves following a standard CTAB extraction protocol and using the DNA as template for the Polymerase Chain Reaction (PCR) for amplification of one genomic regions of the rDNA: region of the nuclear large subunit rRNA gene using the fungal specific primers NL1 and NL4 (O'Donnell, 1992).

PCR products were purified by means of High Pure PCR Product Purification Kit (ROCHE) following the instructions in the manual and sequenced in both directions using a MegaBACE^{TM}1000 DNA Sequencing System. A BLAST comparison of the sequences that were obtained from the white smut fungus from *T. fluminensis* with other sequences of *Kordyana* available in GenBank yielded a 96% homology with *K. tradescantiae* and *K. celebensis*. Also a significant similarity (87-88%) was found with several species of *Exobasidium.* In order to gather additional evidence of the taxonomic distinction of the fungus on *T. fluminensis* from other species in *Kordyana* the sequence generated in this study was compared with LSU sequences from other works (Begerow et al., 2002) downloaded from NCBI and aligned using the Clustal W algorithm in the MEGA program (Tamura et al., 2007). The LSU sequences of the *Anthracoidea arenaria* 1394A.are (Ustilaginomycetidae), were used as outgroups in the analysis. The final LSU alignment contained 24 taxa and 425 aligned nucleotides (Table 3). The aligned sequences were subjected to maximum parsimony and maximum likelihood analyses in PAUP 4.0 b10 (Swofford, 2002), and Bayesian inference and Markov chain Monte Carlo simulation implemented in MrBayes ver 3.0 with three repetitions (Ronquist & Huelsenbeck, 2003). For the maximum likelihood analyses the GTR+I+G model was selected by the program Modeltest (http://bioag.byu.edu/zoology/crandall_lab/modeltest.htm) and Bootstrap analysis was

performed with 1000 random replicates. Bayesian analysis was conducted on the same

aligned dataset after MrModeltest v. 2.2 (Nylander, 2004) was used to determine the

nucleotide substitution model GTR +I+G models. The Markov Chain Monte Carlo

(MCMC) analysis of four chains started with a heating parameter of 0.1 from a random

tree topology and lasted 5,000,000 generations. Trees were saved each 100

generations, resulting in 50,000 saved trees. Burn-in was set at 1,250,000 generations

after which the likelihood values were stationary, leaving 37,000 trees from which the

50 % majority rule consensus trees and posterior probabilities were calculated.

Table 3. List of fungal species whose LSU sequence was included in the phylogenetic analysis

List of specimens studied	
Specimens	***Sequence GenBank accession numbers**
1. Exobasidiomycetidae	
Entyloma dahliae	AY272035.1
Tilletiopsis pallescens	AB178263.1
Kordyana celebensis	AF487401.1
Kordyana tradescantiae	AF487402.1
Exobasidium bisporum	AB177598.1
Eballistra lineata	AF229351
Microstroma album	AF352052.1
Quambalaria pitereka strain QP45	DQ823439.1
Tilletia controversa	DQ832244.1
Doassansia epilobii	AF007523
Entyloma arnoseridis	DQ645528.1
Tilletiopsis washingtonensis	DQ025487.1
2. Ustilaginomicetidae	
Moesziomyces bullatus	DQ831011.1
Sporisorium andropogonis-micranthi	AY740100.1
Sporisorium penniseti	AY740130.1
Ustilago hordei	AF453934.1
Ustilago nuda	AJ236139.1
Tranzscheliella hypodytes	DQ875373.1
Melanotaenium cingens	DQ875364.1
Melanustilospora ari voucher HUV11528	EF517924.1
Urocystis ulei voucher RB5053	EF517930.1
Vankya heufleri voucher HUV 15007	EF653981.1

*List of sequences deposited in NCBI

The maximum parsimony analysis produced eight trees and the maximum likelihood analysis led to the selection of one tree having as 3289.54702 of probability (date not shown). Bayesian analysis yielded a tree having a topology equivalent to that obtained as above (Fig 12). Results obtained through the three phylogeny analysis models, combined with the distinct morphological features of the fungus on *T. fluminensis* have shown that the *K. brasiliensis* indeed represents a new species. This was confirmed by the consistency on the topology of the trees generated for the species of *Kordyana.*

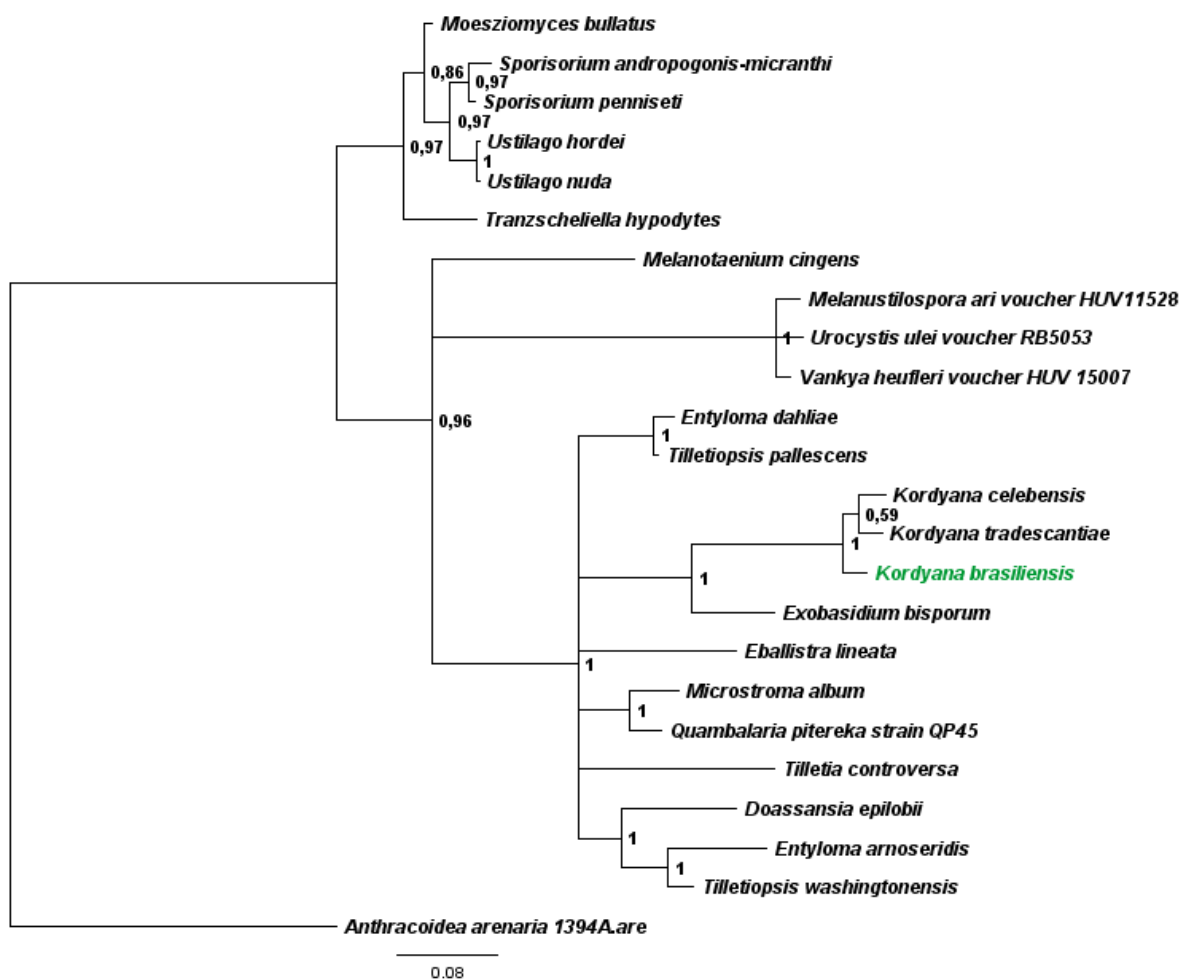**Fig. 12.** The 50% majority rule tree of 37,500 trees obtained from a Bayesian analysis of the LSU sequence alignment. Bayesian posterior probabilities are given at the nodes and the scale bar shows 0.08 expected changes per site. The tree was performed to evaluated the phylogenetics position of *Kordyana brasiliensis* rooted with *Anthracoidea arenaria* 1394Aare.**Pathogenicity and biocontrol potential**

Preliminary pathogenicity experiments were conducted for all the basidiomycetous fungi found, i.e. *Ceratobasidium tradescantiae*, *K. brasiliensis* and *Uromyces commelinae* sp. *Tradescantia fluminensis* plants originating from Brazil or imported from New Zealand (NZ) were used in the pathogenicity experiments. For *K. brasiliensis,* the fungus was cultivated in MNM (Melin-Norkrans Modified Medium) and incubated in the dark at 25 °C. After 10 days, sporidia were collected by pouring 30 ml of sterile water on the culture surface and scraping it with a rubber spatula. The resulting suspension was filtered through four layers of cheese cloth and the final concentration of the suspension was adjusted to 1×10^7 sporidia/ml for inoculation. The cell suspension was sprayed on the leaf surface (abaxially and adaxially) without wounding. After inoculation, 10 plants were covered for 48 hours with plastic bags wetted inside and having water soaked cotton internally and left at room temperature (approximately 25 °C). After that period, the plastic bags were removed and plants were maintained in a greenhouse (26 +/- 2°C) and watered daily. Ten non-inoculated healthy plants, kept under the same conditions served as controls. For the biotrophic fungi *C. tradescantiae* and *Uromyces commelinae*, 10 healthy potted *T. fluminensis* plants imported from NZ were cultivated side-by-side (pots kept 5 cm apart) with diseased plants collected during field surveys. Plants were kept for one year on a shaded bench outdoors and watered regularly.

Another approach, successfully developed in previous biocontrol programmes (Scott *et al.* 2002; Morin *et al.* 2006) involved the selection of strains most infectious to the target. Thus, sourcing *Uromyces* and *Kordyana* strains that were pathogenic the New Zealand biotype of *T. fluminensis.* This technique involved placing sentinels (potted plants of the New Zealand biotype) at ten selected localities where these pathogens occurred on natural populations of *T. fluminensis* in Southern Brazil, namely in Paraná, Santa Catarina and Rio Grande do Sul (Table 4; Fig 13).

Fig. 13. Distribution of sentinel plants at places of occurrence of diseased *Tradescantia fluminensis* in the field in southern Brazil.

These were left in the field for 9 months (left in the field February 2007 and re-collected in December 2007) after which each site was visited. In the second visit to sites sentinel plants were carefully examined and later removed and destroyed if not bearing disease symptoms or otherwise brought back to the base at the Universidade Federal de Viçosa for further examination..

Additionally, after repeated failure in attempts to reproduce the disease with inoculum obtained from pure cultures of the several fungi that were obtained, and after observations were made that healthy *T. fluminensis* plants placed close to infected

plants brought from the field became infected with white smut an experiment was designed taking advantage of the natural spread of the disease that was seen to occur in the shade house in Viçosa. A selection of 70 species was chosen (Table 5) and three potted individuals of each species, including *T. fluminensis* of New Zealand origin, were placed together with a population of *T. fluminensis* that had become naturally infected and presented a high percentage of leaves infected with the fungus. The test-plants were organized in rows on the floor of a shade-house and between each row at a 50 cm distance, a row of pots containing diseased *T. fluminensis* was placed (Fig. 14). During the first month, test-plants were examined weekly for disease symptoms, and at monthly intervals following the first month.

Table 4. List of *Tradescantia fluminensis* sentinel plants (New Zealand stock) left exposed to inoculum of *Uromyces commelinae* and *Kordyana brasiliensis* in the native range resulting infections.

Plant No:	Location	GPS coordinates	Pathogen present*	Recollection (Y/N)
1	São Francisco de Paula plant a	S 28° 23' 45.9"; W 049°32' 49.0"	K	Y – No disease
2	São Francisco de Paula plant b	S 28° 23' 45.9"; W 049° 32' 49.0"	K	Y – No disease
3	Bento Gonçalves Rs 444 plant a	S 29° 11' 08.8"; W 051° 36' 08.1"	U	Y – *Uromyces* infection
4	State border Rio Grande do Sul & Santa Catarina Br 116	S 28° 14' 6.3"; W 050° 46' 4.7"	K/U	Y - No disease
5	Bento Gonçalves Rs 444 plant b	S 29° 11' 08.8"; W 051° 36' 08.1"	U	Y – No disease
6	Lages plant a	S 27° 47' 26.8"; W 050° 21' 14.5"	K/U	Y – No disease
7	Lages plant b	S 28° 14' 6.3"; W 050° 46' 4.7"	K/U	Y – No disease
8	Serra do Rio do Rastro	S 28° 23' 45.9" ; W 049° 32' 49.0"	K	N – plant not recovered due to site flooding

* **K** = Sentinel plants placed amongst/proximate to plants infected by *K. brasiliensis*

* **U** = Sentinel plants placed amongst/proximate to plants infected by *U. commelinae*

Table 5. List of test-plants included in the specificity test

BOTANIC FAMILY	PLANT SPECIES
Amaranthaceae	*Alternanthera philoxeroides* (Mart.) Griseb
Alismataceae	*Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli
	Sagittaria montevidensis Cham. & Schltdl.
Anacardiaceae	*Mangifera indica* L.
	Pistacia vera L.
	Rhus copallinum L.
	Schinus molle L.
	Schinus terebinthifolia Raddi
	Spondias purpurea L.
Araceae	*Pistia stratiotes* L.
	Anthurium sp.
Arecaceae	*Rhopalostylis sapida* H. Wendl. & Drude
Asteraceae	*Emeranthus* sp.
Balsaminaceae	*Impatiens walleriana* Hook. f.
Bixaceae	*Bixa orellana* L.
Bromeliaceae	*Aechmea aquilega* (Salisb.) Griseb.
Chrysobalanaceae	*Licania tomentosa* (Benth.) Fritsch
Commelinaceae	*Callisia repens* (Jacq.) L.
	Callisia warszewicziana (Kunth & Bouché) D.R. Hunt
	Commelina benghalensis L.
	Commelina diffusa Burm. f.
	Commelina erecta l.
	Dichorisandra thyrsiflora J.C. Mikan
	Gibasis schiedeana (Kunth) D.R. Hunt
	Siderasis fuscata (Lodd.) H.E. Moore
	Tradescantia fluminensis Vell.
	Tradescantia pallida (Rose) D.R. Hunt
	Tradescantia spathaceae Sw.
	Tradescantia zebrina Heynh.
	Tradescantia zononia (L.) Sw.
	Tripogandra diuretica (Mart.) Handlos.
	Tinantia sp.
Costaceae	*Costus erythrophyllus* Loes
Cyperaceae	*Cyperus rotundus* L.
Euphorbiaceae	*Euphorbia heterophylla* L.
	Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg.
Fabaceae	*Cassia grandis* L. f.
Faboideae	*Dalbergia* sp.
Juncaceae	*Juncus* L.
Malvaceae	*Theobroma cacao* L.
Maranthaceae	*Maranta bicolor* Ker Gawl.
Melastomataceae	*Tibouchina herbaceae* (DC.) Cogn.
	Miconia calvescens DC.
Meliaceae	*Cedrela fissilis* Vell.
	Cedrela odorata L.

Table 5. (continued)

BOTANIC FAMILY	PLANT SPECIES
Myrtaceae	*Eugenia uniflora* L.
	Plinia edulis (Vell.) Sobral
	Plinia trunciflora (O. Berg) Kausel
	Psidium cattleianum Sabine
	Psidium guava Griseb
	Syzygium cumini (L.) Skeels
Pontederiaceae	*Eichornia crassipes* Solms
Rosoideae	*Rubus fruticosus* L.
Rosaceae	*Prunus persica* (L.) Batsch
	Rosa sp.
Rubiaceae	*Coffea arabica* L.
Rutaceae	*Citrus x limon* (L.) Osbeck
Solanaceae	*Solanum lycocarpum* A. St.-Hil.
Strelitziaceae	*Strelitzia reginae* Aiton
Thyphaceae	*Typha domingensis* Pers.
Verbenaceae	*Lantana camara* L.
	Verbena sp.
Zingiberaceae	*Hedychium coronarium* J. König
	Zingiber officinale Roscoe

More recently a new demonstration of pathogenicity of *K. brasiliensis* was performed involving the selection of leaves bearing sporulating white smut lesions that were attached to the vaseline-coated inner side of empty Petri dish lids that were placed on a 19 cm high metal tripod. above healthy *T. fluminensis* plants. This apparatus was kept in a dew chamber for 48 hours at 25 ± 2°C in order to allow basidiospores ejected from infected leaves to land on the underlying plants. . Plants were then transferred to a greenhouse and examined at three-day intervals.

The study involving the use of sentinel plants had as its main aim to find rust strains capable of infecting the New Zealand *T. fluminensis* biotype for the purpose of developing a classical biocontrol agent for the plant. After 9 months, infection was observed at only one of the sites (Vale dos Vinhedos, Bento Gonçalves, Rio Grande do Sul) (Fig. 14). Unfortunately, similar to what had been previously observed for uprooted plants that were brought with rust infection to Viçosa from Southern Brazil, disease on potted sentinel plants slowly vanished as new leaves were produced. Interventions

such as transferral of urediniospores onto healthy leaves and keeping the plants in controlled temperature rooms under lower temperatures failed.

A set of 11 selected species of Commelinaceae (*Callisia repens, C. warszewicziana, Commelina benghalensis, C. diffusa, C, erecta, Dichorisandra thyrsiflora, Tradescantia fluminensis, T pallida, T. Spathaceae, T. zebrina and Tripogandra diuretica*) was taken from Viçosa to three localities, namely: Serra do Espigão, BR 116 (Km 108), close to a shrine (state of Santa Catarina),Vale dos Vinhedos, Bento Gonçalves (state of Rio Grande do Sul) and at Cascata, EMBRAPA Clima Temperado, Pelotas (state of Rio Grande do Sul)na These plants were left in the vicinity of naturally rust-infected *T. fluminensis* plants as an attempt to access host-specificity of the rust in field conditions. This was conducted as an attempt to overcome the persistent difficulties of keeping and manipulating *U. commelinae* under controlled conditions. The sites were visited 8 months later. At the Serra do Espigão site no rust infection appeared on the test plants and at the Vale dos Vinhedos site (where a sentinel plant of the NZ biotype became infected previously) all plants were either stolen or destroyed... . The plants left in the research station in Pelotas (Rio Grande do Sul) were seemingly, contrarily to our request, kept in a greenhouse and the infected plants got cured without the disease having been transmitted to any neighbouring commelinaceous plant.

Other studies on the rust that would occur in the next stage such as evaluation of host-specificity and elucidation of the fungus life cycle were temporarily abandoned because of such practical difficulties. It is not possible, at this stage, to determine the biocontrol potential of *U. commelinae*.

Fig. 14. A sentinel plant that became rust infected after being left in the field at the site at Vale dos Vinhedos (Bento Gonçalves, state of Rio Grande do Sul).

The indirect host-range test has demonstrated that *K. brasiliensis* was highly specific to *T. fluminensis*. Healthy *T. fluminensis* from New Zealand-origin plant stock showed "white smut" symptoms one month after they were placed in the vicinity of plants infected with *K. brasiliensis* in the shade house. None of the other test plants belonging to the other 69 taxa became infected by *K. brasiliensis* after 12 months of observation (Fig. 15) .

Fig 15. A-C Centrifugal phylogenetic host-range test of *Kordyana brasiliensis.* A-General view of experiment in shade house. B- Test-plants growing intermixed with infected *Tradescantia fluminensis.* C- Close-up showing a *T. fluminensis* individual heavily infected with white smut growing among test-plants.

The method of inoculation through basidiospore drop from overlying infected leaves was successful at yielding typical white smut symptoms and *K. brasiliensis* sporulating colonies on test-plants (Fig. 16).
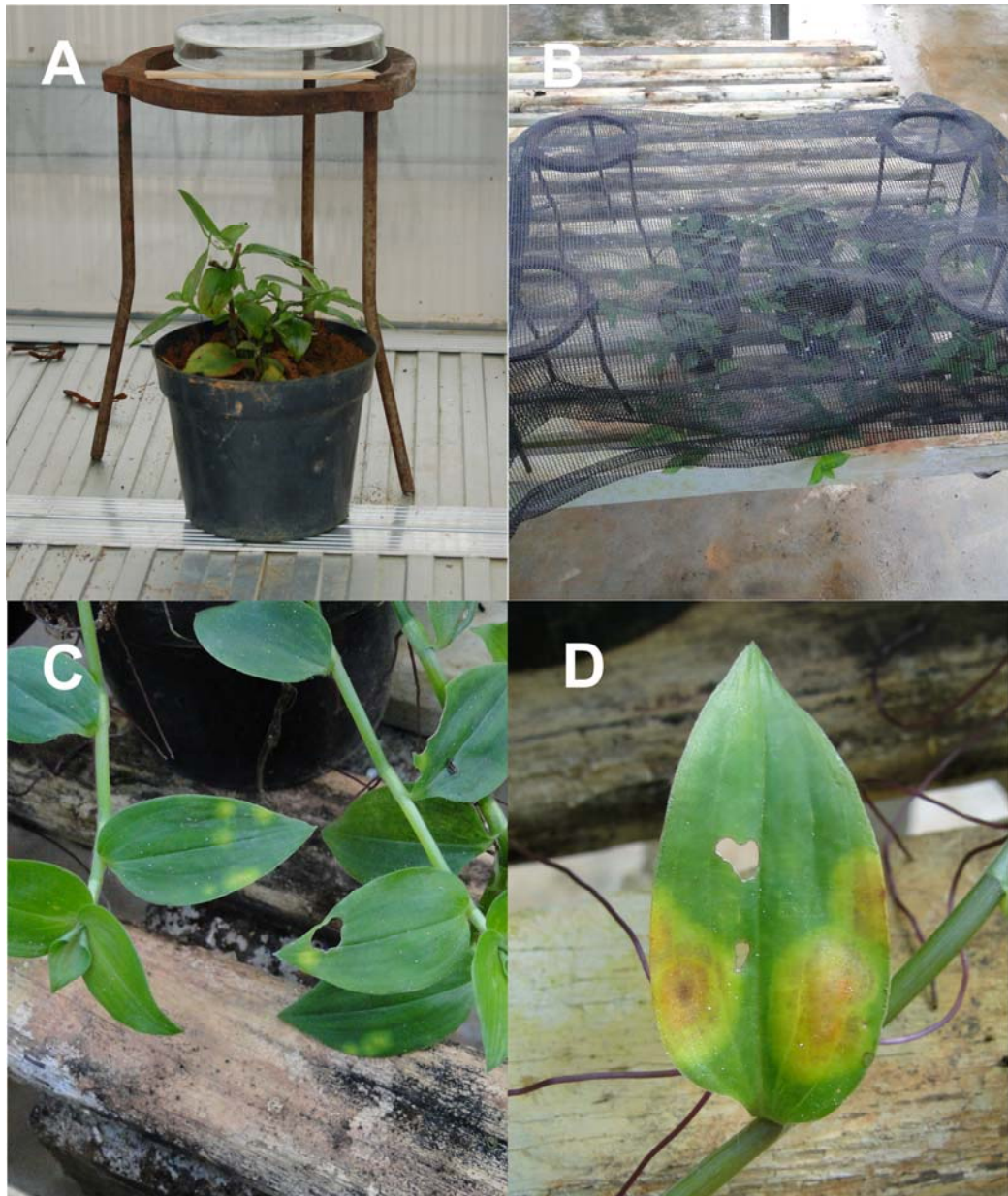
Fig. 16. *Kordyana brasiliensis* inoculation through ejection of basidiospores from infected leaves. A- Basidiospore ejection apparatus. B- Plants immediately after inoculation. C-D- White smut symptoms resulting from basidiospore drop.

Kordyana brasiliensis and *Uromyces commelinae* are for the first time reported on *T. fluminensis* in Brazil. The genera *Kordyana* has been reported on *T. fluminensis* only from Ecuador (Petrak, 1950) and Costa Rica (Gómez and Kisimova-Horovitz, 1997) and no rusts had been reported on *T. fluminensis* in Brazil (Hennen et al. 2005). Although in some field situations these biotrophic basidiomycetes did not cause severe disease symptoms on *T. fluminensis,* on other occasions (particularly in shaded

situations) damage was significant. Diseased plants appeared weakened and defoliated as compared to healthy *T. fluminensis* plants. All but one species of *Kordyana* spp. are known to attack plants belonging to the Commelinaceae. Our results indicate that *Kordyana brasiliensis* is a highly host-specific pathogen of *T. fluminensis* but even if it infected other members of the Commelinaceae this would probably not represent a limitation for its use as a biological control agent in New Zealand since there are no native plants, nor any relevant crop plants, belonging to this plant family. The other basidiomycete, *Ceratobasidium* sp. caused no significant damage in the field. It still remains unclear whether the mild blight symptoms appearing on colonized leaves only represent naturally senescent leaves or become necrotic because of the fungus infection. This species does not appear to deserve further consideration as a possible candidate agent. It is nevertheless interesting to note that no *Ceratobasidium* species have previously been reported on the genus *Tradescantia* and no other species in this genus has ever been reported as a foliar biotroph (Roberts, 1999). Based on the morphological characteristics observed during this study, *Ceratobasidium* sp. was recognized as a new species and will be described separately.

Colletotrichum includes some of the best known examples of the use of fungal pathogens as weed biocontrol agents (Bailey & Jeger, 1992). Some of the best known mycoherbicides involved the use of species of propagules of *Colletotrichum* as active ingredients such as in COLLEGO (Bowers, 1986) and BIOMAL (Mortensen & Mokowski, 1997). There are also examples of use of fungi in this group as classical biocontrol agents (Barreto et al., 2001). *Colletotrichum falcatum* is a common pathogen of grasses and other monocots. The only reports of *Colletotrichum* on members of the Commelinaceae are those of *C. falcatum* associated with *Dichorisandra* sp. and *Zebrina pendula* Schinzl. in Florida. On *T. fluminensis*, there is only one record of an undetermined *Colletotrichum* sp. in Flórida and Texas (Farr & Rossman, 2010), but the pathogenicity status of these is obscure. Although somehow different from *C. falcatum* as described in Bailey & Jeger (1992) we decided to temporarily identify it with that

epithet. Preliminary pathogenicity studies did not confirm its pathogenicity to the biotype of *T. fluminensis* from New Zealand although its pathogenicity to plants grown from the population from which it was obtained was confirmed.

Cercospora apii and *Mycospherella tradescantiae* caused severe necrotic disease symptoms on leaves and stems of *T. fluminensis* in the field. Crous and Braun (2003) listed numerous hosts belonging to many distinct plant families for *C. apii*, however, this is the first report of *C. apii* on *T. fluminensis*. Despite its supposedly wide host range, we are planning to conduct host range tests based on the centrifugal phylogenetic method (Wapshere, 1974) to evaluate the specificity of this isolate of *C. apii*. It is known that specificity can exist in populations within a fungal species known to have a wide host range (Barreto et al., 2001b; Pereira et al., 2003). In case this isolate will prove to be host-specific, *C. apii* may deserve further consideration for use in classical biological control.

No *Mycosphaerella* spp. was ever been reported attacking members of the Commelinaceae (Aptroot, 2006). A study of the morphology of *Mycosphaerella* sp. from *T. fluminensis* indicates that this is a new species which will be described elsewhere. As it causes severe necrotic disease symptoms on *T. fluminensis* and members of *Mycosphaerella* are often host-specific, this fungus may be a promising candidate for the classical biological control of *T. fluminensis*.

Rhizoctonia solani is known to be a highly polyphagous pathogen having more than 2500 hosts throughout the world (Farr & Rossman, 2010) causing "damping-off", seed and root rots and blight of aerial parts (Garibaldi et al., 2009a; 2009b). In the only occasion when this fungus was found attacking *T. fluminensis* the plants were growing in fully exposed conditions and it was thought that the disease was in fact foliage scalding due to excess light. Although all specimens brought to the lab bear fungus colonies, it is still possible that there is a connection between sunlight damage and the disease. It is clear that *R. solani* has no potential for use in classical biocontrol.

Similarly to what has been observed for *R. solani* the sole occurrence of *Sclerotium rolfsii* was under abnormal conditions under very wet conditions in the greenhouse. It is also a highly polyphagous pathogen of no interest for classical biocontrol. This fungus was known until now only on the following Commelinaceae: *Dichorisandra* sp. – in Flórida, and *Zebrina pendula* Schinzl – in Hawaii (Farr & Rossman, 2010; Mendes & Urben, 2010).

The results of the pathogenicity studies clearly demonstrated that *K. brasiliensis* is highly host-specific to *T. fluminensis* and capable of causing a severe disease on plants of the biotype present in New Zealand. No species other than *T. fluminensis* was attacked in the field by this fungus or infected when directly exposed to inoculum under closer scrutiny. Such a level of host-specificity, although desirable could be regarded as unnecessary in the case of New Zealand since there is no native or cultivated plants of relevance in this family in the country. Potential for its use as a classical biocontrol agent is regarded as very high and, considering the weather similarities and equivalent latitude of the natural range of *K. brasiliensis* in southern Brazil and the regions invaded by *T. fluminensis* in New Zealand, it is probable that the fungus will adapt well if introduced. Such climatic and latitudinal matching are commonly regarded as good indicators of biocontrol candidates successfully adapting to the new range (Wapshere, 1985). Colonization by such fungi generates sinks for plant reserves and often lead, in the case of crop plants, to major reductions in productivity and, in the case of plants in natural habitats, to loss of the ability to compete with other plants (Watson, 1991; Charudattan, 2005). One of the most important pre-requisites for using an organism as a biocontrol agent is a high-host specificity, a feature that is recognized as occurring at the highest level in biotrophic fungi such as *K. brasiliensis* (Frank, 1992). The affinity of *Kordyana* with *Entyloma* and similarity of the disease as that caused by *Entyloma* (white smut) may also be regarded as indications that a similar level of success might be achieved with the introduction of *K. brasiliensis* as that obtained after the introduction of *Entyloma ageratinae* R.W. Barreto & H.C. Evans against mist-flower *Ageratina riparia* in

Hawaii in the 1970s (Trujillo, 2005) and in New Zealand in the 1990s (Fröhlich et al., 2000). Results of both releases have been reported as spectacular in both cases (Trujillo, 2005; Barton et al., 2007). In New Zealand evaluations of the results of the introduction of white smut on *A. riparia* four years after its introduction have shown that the fungus was able to disperse to distances superior to 400 Km from release sites, establishing stable populations where introduced, significantly reducing weed infestations and contributing to recovery of local biodiversity (Barton et al., 2007).

The bacterium collected in the state of Rio de Janeiro was identified as *Burkholderia andropogonis* and its pathogenicity was demonstrated. Inoculated plants of the biotype brought from New Zealand were highly susceptible to this pathogen and plant death commonly resulted from inoculations. Unfortunately, host-range tests indicated that, although restricted to monocots this bacterial isolate was capable of infecting all eight species of the Commelinaceae included in the test plus species in five additional families, i.e. pineapple (Bromeliaceae), Eriocaulaceae, maize and sorghum (Poaceae), cattail (Typhaceae) and ginger (Zingiberaceae). Although *B. andropogonis* is not known to be a pathogen of maize or sorghum in Brazil, further studies are needed to fully clarify the risk represented by *B. andropogonis* to crop and non-crop plants. Until then, its potential for introduction into New Zealand or other regions of the world or its use as a bioherbicide can not considered any further.

Until now only a limited area of the Neotropics was surveyed for *T. fluminensis* pathogens. It is expected that the continuation of the surveys with the expansion to new areas of natural occurrence of *T. fluminensis* will result in new additions to this list of pathogens.

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