Pathogens from Brazil for classical biocontrol of *Tradescantia fluminensis*

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Summary

Tradescantia fluminensis Vell., also known as wandering Jew, is an herbaceous monocot native to South America. It is an invasive plant in New Zealand and the south-eastern United States where it is considered highly invasive by the Florida Exotic Pest Plant Council. The pathobiota of *T. fluminensis* in Brazil is almost unknown and could include phytopathogenic microorganisms that could be used in classical biological control programs. A survey for specialized, coevolved phytopathogenic microorganisms of *T. fluminensis* was initiated in 2003. Five fungal species have been collected including three basidiomycetes—a rust fungus (*Uredo* sp.), *Kordyana tradescantiae* (Pat.) Racib. and *Ceratobasidium* sp.; a hyphomycete—*Cercospora apii* Fresen. and an ascomycete—*Mycosphaerella* sp. A bacterial disease was also observed and the bacterium identified as *Burkholderia andropogonis* (Smith, 1911), based on morphological, biochemical and molecular methods. Its pathogenicity to *T. fluminensis* was confirmed, and a host-range test was performed. Unfortunately, results indicated that the bacterium is not sufficiently host-specific for classical introductions. Observations of the damage caused by fungal pathogens in the field suggest that those with the best potential as biological control agents are *Uredo* sp., *K. tradescantiae* and *Mycosphaerella* sp.

Keywords: classical biological control, invasive weed, plant disease.

Introduction

Tradescantia fluminensis Vell. (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 abundant 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 ranked among the most invasive species of Florida (FLEPPC, 2003) and 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, 2004; Standish, 2002). It has no significant natural enemies (arthropods or pathogens) in New Zealand (Winks *et al*., 2003). Surveys of fungal pathogens of native weeds in Brazil have yielded a plethora of potential biological control agents over the years (Barreto *et al*., 1995. 1999a,b; Barreto and Evans, 1994; Barreto and Torres, 1999; Monteiro *et al*., 2003; Pereira and Barreto, 2000, 2005; Soares and Barreto, 2006; Seixas *et al*., 2007). Two of the fungal pathogens found during these surveys have been introduced from Brazil into other parts of the world, namely: *Prospodium tuberculatum* (Speg.) Arthur for the biological control of *Lantana camara* L. (Barreto *et al*., 2001a; Ellison *et al*., 2006) in Australia and *Colletotrichum gloeosporioides* f.sp. *miconiae* for the biological control of *Miconia calvescens* Schrank and Mart. ex DC. in Hawaii (Barreto *et al*., 2001b). Although Brazil is considered to be the centre of origin of *T. fluminensis*, there is not a single pathogen recorded to be associated with this plant species in this country in the world literature (Table 1). A cooperative research project recently initiated between the Universidade Federal de Viçosa (Brazil) and Manaaki Whenua Landcare Research New

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Table 1. Fungal pathogens recorded on *Tradescantia fluminensis* in the literature (Petrak, 1950; Gómez and Kisimova-Horovitz, 1997; Waipara, 2006; Farr *et al*., 2007).

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

Zealand Ltd. is aimed at surveying and evaluating the native pathobiota associated with *T. fluminensis* in Brazil for potential classical biological control agents. This paper gives a preliminary account of the pathogens found during these surveys and their potential as classical biological control agents for *T. fluminensis*.

Materials and methods

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 and December 2005: 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 spores 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.

Preliminary pathogenicity experiments were conducted for all the basidiomycetous fungi found, i.e. *Ceratobasidium* sp., *K. tradescantiae* (Pat.) Racib. and *Uredo* sp. *T. fluminensis* plants originating from Brazil or imported from New Zealand (NZ) were used in the pathogenicity experiments. To verify the pathogenicity of *K. tradescantiae*, the fungus was cultivated in Melin-Norkrans modified medium (MNM) 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 h 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 *Ceratobasidium* sp. and *Uredo* sp., ten 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 1 year on a shaded bench outdoors and watered regularly.

Results

Five fungal species and a bacterium, collected in four different states, were found associated with diseased *T. fluminensis*: three basidiomycetes—a rust fungus (*Uredo* sp.), *K. tradescantiae*—causing white smut-like symptoms and the blight-causing-fungus *Ceratobasidium* sp.; a leaf-spot- and stem necrosis-causing hyphomycete—*Cercospora apii* Fresen. and an ascomycete that is associated with leaf-spots—*Mycosphaerella* sp. (Table 2). The phytopathogenic bacterium was identified as *Burkholderia andropogonis* (Smith, 1911). Three of the fungal pathogens were isolated in culture: *K. tradescantiae*, *C. apii* and *Mycosphaerella* sp. Repeated attempts to isolate *Ceratobasidium* sp. associated with leaf blight were unsuccessful. We believe that this fungus is in fact a biotroph, since often even a complete colonization of the abaxial surface of leaves

Table 2. Fungal pathogens found on *Tradescantia fluminensis* during field surveys in Brazil.

Fungal species	Distribution in Brazilian states[a]
Ceratobasidium sp.	SC (1); RS (3)
Cercospora apii	MG (2); SC (1); PR (2)
Mycosphaerella sp.	RS (1)
Uredo sp.	PR (1); SC (1); RS (2)
Kordyana tradescantiae	PR (3); SC (3); RS (9)

[a] The numbers in parentheses represent how many times each fungus was collected in a state (*MG* Minas Gerais; *PR* Paraná; *RS* Rio Grande do Sul; *SC* Santa Catarina).

(easily observed by an extensive external coverage of the tissues by a mycelial mat) was not accompanied by any sign of necrosis. Attempts to isolate *K. tradescantiae* on vegetable broth-agar (VBA) were 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) but also failed to promote any fungal growth. *K. tradescantiae* was finally successfully isolated on MNM, a culture medium commonly used for ectomycorrhizal basidiomycetous fungi (Marx, 1969).

No disease symptoms were observed on plants from Brazil or NZ inoculated with *K. tradescantiae*, and no symptoms were observed to spread when placing Brazilian plants infected by *Uredo* sp. and *Ceratobasidium* sp. beside healthy NZ plants after over 1 year of observation.

Discussion

Kordyana tradescantiae and *Uredo* sp. are reported for the first time on *T. fluminenis* in Brazil. *K. tradescantiae* 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 caused no severe disease symptoms on *T. fluminensis*, on other occasions (particularly under heavily shaded areas), damage was significant. Diseased plants appeared weakened and defoliated as compared to healthy *T. fluminensis* plants. It seems that both fungalspecies are promising candidates for the classical biological control of *T. fluminensis* (Table 3). Although *K. tradescantiae* is known to attack plants belonging to several genera in the Commelinaceae, this is not a limitation for its use as a biological control agent in New Zealand where there is not any native plant nor any crop plant of relevance belonging to this family. The other basidiomycete, *Ceratobasidium* sp., caused no significant damage in the field. It still remains unclear whether the slight 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 (Table 3). It is nevertheless interesting to note that no *Ceratobasidium* species has previously been reported on the genus *Tradescantia*, and no other species in this genus was ever reported as a foliar biotroph (Roberts, 1999). Based on the morphological characteristics observed during this study, *Ceratobasidium* sp. was recognized as a new species that will be described separately.

C. apii and *Mycosphaerella* sp. 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 prove to be host-specific, *C. apii* may deserve further consideration for use in classical biological control.

No *Mycosphaerella* spp. was ever reported attacking members of *Tradescantia*. A study of the morphology

Table 3. Characteristics of fungal pathogens found on *Tradescantia fluminensis* during field surveys in Brazil.

Fungal species	Disease	Damage to host	Likely specificity	Cultured	Biological control potential
Ceratobasidium sp.	Faint leaf-blight	insignificant	High	No	Low
Cercospora apii	Leaf-spot	Significant	Non-specific	Yes	Uncertain
Mycosphaerella sp.	Leaf-spot	Significant	High	Yes	High
Uredo sp.	Rust	Significant	High	No	High
Kordyana tradescantiae	'White smut'	Significant	Low (on Commelinaceae)	Yes	High

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 is being considered as a promising candidate for the classical biological control of *T. fluminensis* (Table 3).

The preliminary pathogenicity tests with *K. tradescantiae*, *Uredo* sp. and *Ceratobasidium* sp. were unsuccessful. No disease symptoms were reproduced through artificial inoculation (with *K. tradescantiae*). Perhaps sporidia of this fungus produced in culture are non-infective, and the fungus relies on basidiospores as an infective stage or, perhaps, the fungus loses pathogenicity when cultivated. Nevertheless, it was observed that this disease was naturally transmitted from diseased to healthy plants in Viçosa. This did not happen with either *Uredo* sp. or *Ceratobasidium* sp.

It was observed that plants infected with *Uredo* sp. and *Ceratobasidium* sp., brought from the field in Southern Brazil, were gradually cured of infection along the months after being cultivated under Viçosa conditions at a lower latitude. This suggests that those two fungal species depend on a cooler climate to produce new infection cycles and, therefore, to preserve their populations.

The bacterium collected in the state of Rio de Janeiro was identified as *B. 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. pinnaple (Bromeliaceae), *Paepalanthus macrocephalus* (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 cannot be 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 still-limited list of pathogens. In addition, the potential for biological control of the pathogens already found is being evaluated. One ongoing study aims at checking the susceptibility of the New Zealand biotype of *T. fluminensis* to the rust and to *K. tradescantiae.* Stations with sentinels (potted plants of the New Zealand biotype) were established at selected places where these pathogens occur on native populations of *T. fluminensis* in Southern Brazil. These will be visited at 3-month intervals for inspection of natural attack by pathogens or arthropods.

Genetic studies are also underway to both clarify the clonal status of *T. fluminensis* in New Zealand as well as compare some DNA regions of the NZ biotype with those found in the native Brazilian range. Chloroplast DNA from over 40 plant specimens from different regions of Brazil are being sequenced to obtain information on the plants' lineages that may help to locate where in Brazil the NZ biotype has originated. These results may then be used to source suitable biotypes of each pathogen agent.

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