Biological control of *Tradescantia fluminensis* with pathogens report August 2011

Robert W. Barreto[1]
Davi M. Macedo[1]

[1] Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, 3657-000, Brazil

Host-specificity of *Kordyana brasiliensis*: test involving direct basidiospore ejection on species of *Commelinaceae*

In order to confirm the high host-specificity indicated by the results obtained in the indirect host-range test for *K. brasiliensis*, as described in the previous report, and also in order to overcome the limitations imposed by the lack of infectivity of fungal strutures of this species produced in culture, a new methodology for host-specificity evaluation was used for a more direct test. This involved gathering *T. fluminensis* leaves naturally colonized by *K. brasiliensis* in the shade house near the lab at Viçosa (Minas Gerais), and attaching them to a sheet of glass coated with vaseline leaving the sporulating abaxial side exposed and placed above test-plants. The sheet of glass was placed 60 cm. above healthy test plants of each species (listed in Tab. 1) in a dew chamber for 48 hours at 22^{o}C +/- 3^{o}C and then transferred to benches in a greenhouse at 25°C ± 2^{o}C (Fig 1). Controls consisted of healthy plants of *T. fluminensis* (biotype from New Zealand) that were either exposed to basidiospore drop together with the other Commelinaceae (positive control) or kept free of inoculation (negative control). Plants were observed weekly for the appearance of symptoms. .

After 18 days of inoculation symptoms appeared on *T. fluminensis* but not in any other species (Fig. 2). The situation remained unchanged until the last evaluation, 65 days after inoculation.

Table 1. List of species of Commelinaceae included in the host-specificity test and results of inoculations after 65 days of observation (+ = infected, - = not infected)

PLANT SPECIES	SYMPTOMS
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	-

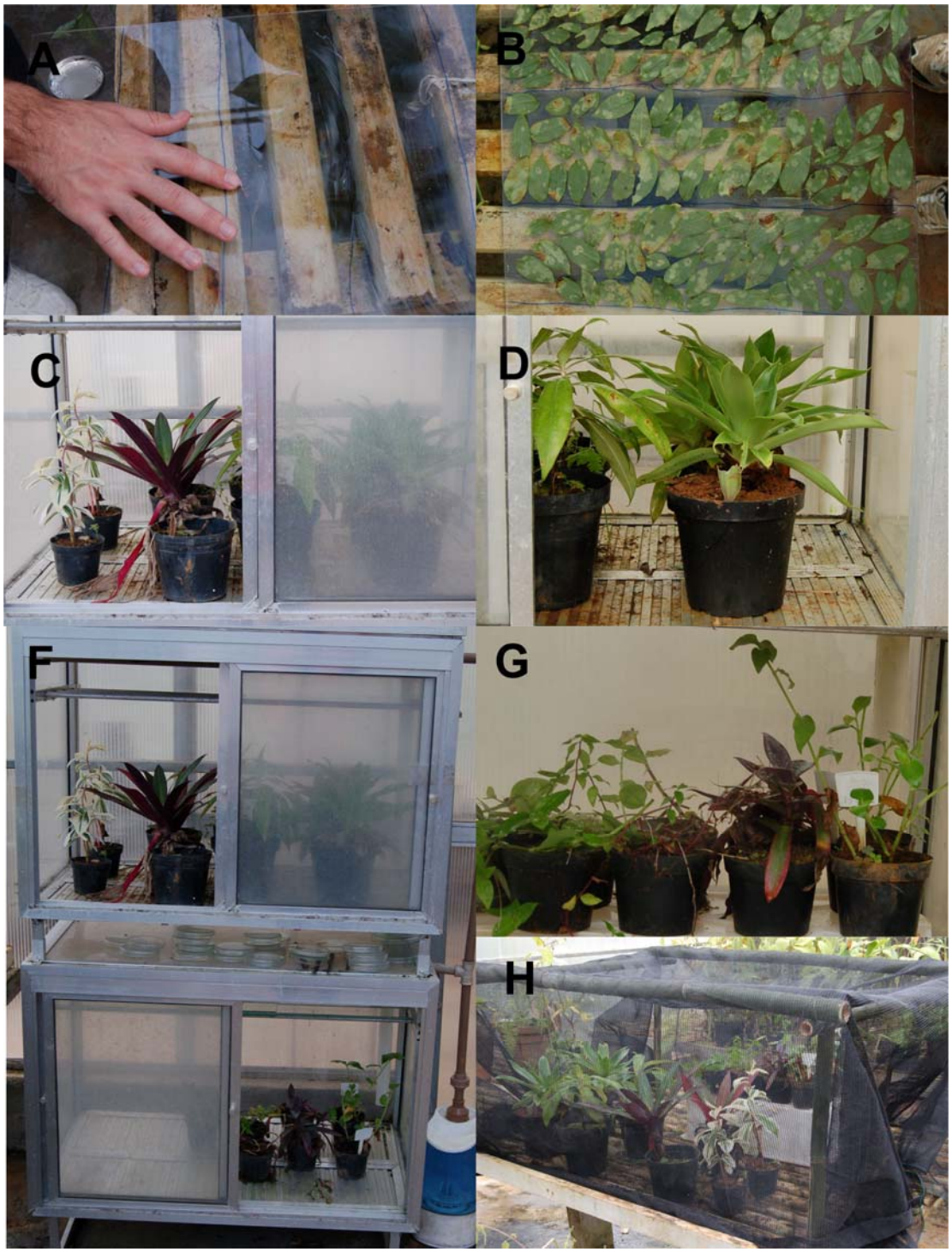

Fig 1. Stages of methodology used in the host-range test of *Kordyana brasiliensis* by direct deposition of basidiospores on test-plants.

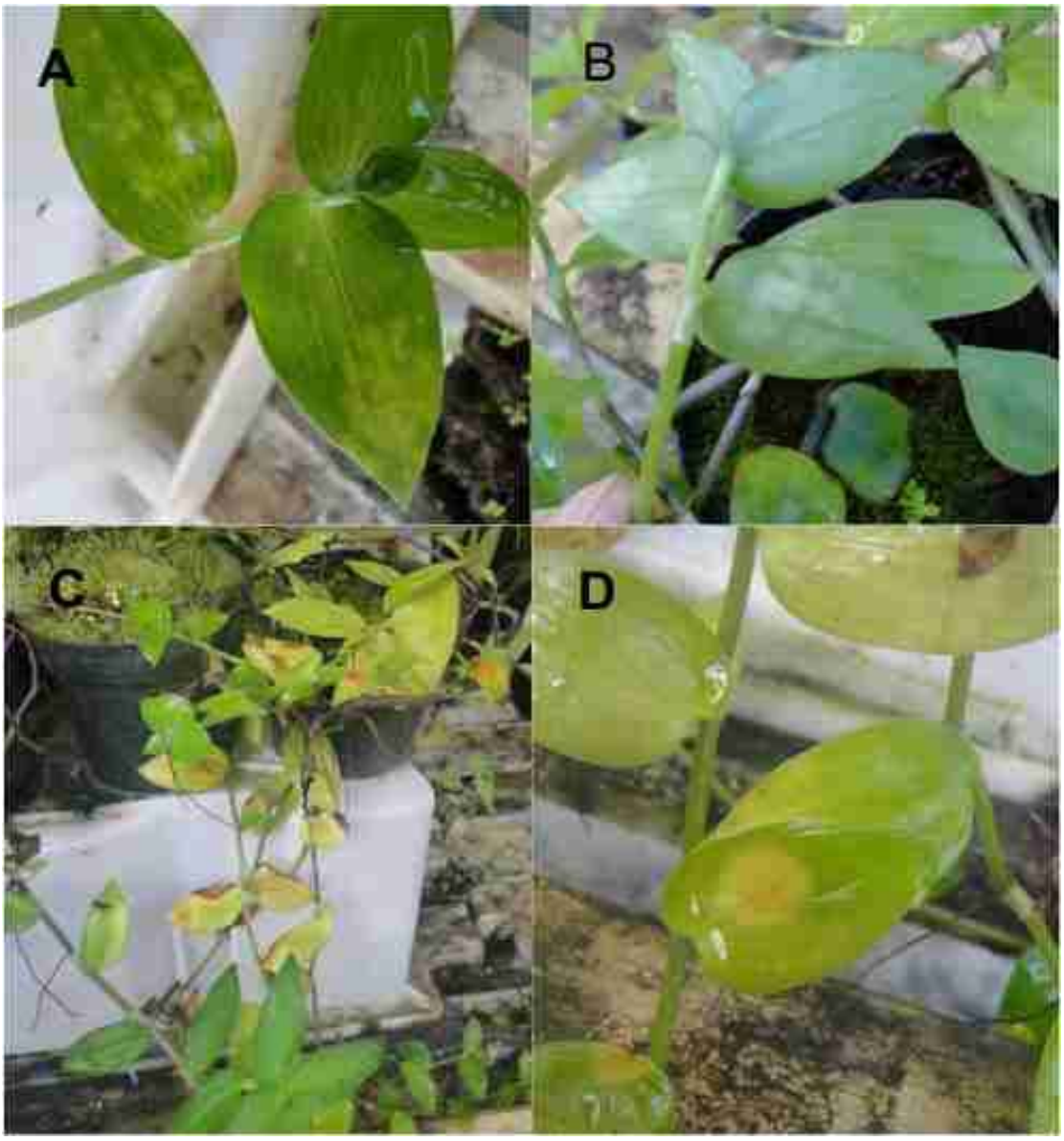

Fig 2. A-B. Appearance of white smut symptoms on*Tradescantia fluminensis* caused by spore-drop of *Kordyana brasiliensis* 18 days after inoculation**. C-D.** Diseased *T. fluminensis* plants 65 days after inoculation.

Viability of inoculum in a selected potential procedure of transportation of *Kordyana brasiliensis* for introduction into New Zealand

As *K. brasiliensis* structures produced *in vitro* were not infective, and basidiospores are too small and fragile for preservation, the remaining practical method for inoculum transportation allowing for introducing the fungus as a classical biocontrol agent in areas where *T. fluminensis* became invasive appears to be through delivery of the fungus as actively growing colonies on living tissues of *T. fluminensis.* As senescence of detached leaves occurs relatively fast for *T. fluminensis* and a period of several days would be required in order to cover the distance between Brazil and New Zealand, ideally, potted plants infected by the fungus should be used for transportation of the fungus. Nevertheless, considering the quarantine restrictions forbidding soil movement between countries, a compromise was attempted. Procedures were suggested by H. C. Evans, based on his experience with the transportation of the white smut fungus of *Ageratina riparia* (*Entyloma ageratinae*) from Mexico into the UK (CABI), and an adaptation of his procedures was used.

A test was performed involving *K. brasiliensis*-infected

The test was performed aimed at verifying sporulation and infectivity of spores after different periods of time under conditions simulating those of transportation of the inoculum source. Twenty freshly harvested 50 cm long stems bearing *K. brasiliensis* infected leaves were selected. The stems that were chosen beared new roots and all stems parts and roots that had direct contact with soil were discarded. Stems and roots were carefully washed. Only healthy leaves or leaves bearing fresh colonies of *K. brasiliensis* were kept on the stems (all senescing leaves or leaves appeared to be damaged by other causes were removed) - and the stems were placed inside new transparent plastic zip-lock bags (27 x 29 cm). Four sheets of paper tissue wetted in sterile water were placed wrapping the base of the stems in order to avoid desiccation of the samples and each bag was sealed. The bags were left at room temperature on a laboratory bench and at 48 hour-intervals the bags were opened and 40 leaves bearing symptoms were detached from stems. The lesions on each infected leaf were cut out with scissors and the infected fragments were attached to the underside of 90.0 mm diam Petri plate lids, as described above for the host-range test. Four apparatus for basidiospore discharge of *K. brasiliensis* onto healthy *T. fluminensis* plants (as illustrated in Fig. 3) were assembled and left in a dew chamber for 48 hours, as described above. Additionally to test plants, one microscope slide was also left under the Petri dish lids of each apparatus for later observation of spore discharge/spore drop and germination events. Evaluations of spore discharge, spore germination and infectivity of inoculum were performed on samples obtained from material

incubated in the plastic bags for periods of 2, 4, 6 and 8 days. Germination was evaluated by depositing a drop of lactophenol centrally on the microscope slide and placing a 3.24 cm^2 cover-slip on it and counting 300 basidiospores deposited on that area in each slide through observation under a light microscope. Each spore was listed as either germinated (bearing a germ-tube with a length of at least the diameter of the basidiospore) or non-germinated. The percentage germination was calculated for the total of the three slides and a mean germination percentage was calculated.

Spore drop was abundant under samples representing all four periods of time. Basidiospore germination average was around 60% for all treatments. As evaluation progressed it was observed that the number of spores belonging to species other than *K. brasiliensis* dropping from the samples of infected *T. fluminensis* also increased. Infectivity was kept only for periods of 2 and 4 days of incubation within the bags. For those treatments, symptoms appeared 23 days after inoculation. It was noticed that at periods of 6 and 8 days of incubation the growth of a combination of saprophytes and mycoparasites was intense and possibly harmful for the infectivity of spores produced on the lesions provoked by *K. brasiliensis.*

Although preliminary, this essay yielded result that indicate that, although transportation of inoculum of *K. brasiliensis* into New Zealand is feasible, it will require the fastest possible method of transportation. Use of ordinary post is ruled out and use of express /courier services such as DHL is regarded as risky as they require at least 5-6 days to deliver the samples to Landcare (Auckland), starting from Rio de Janeiro. It appears that hand-carrying the specimens from Viçosa would be the most appropriate method of transportation in the case of this fungus.

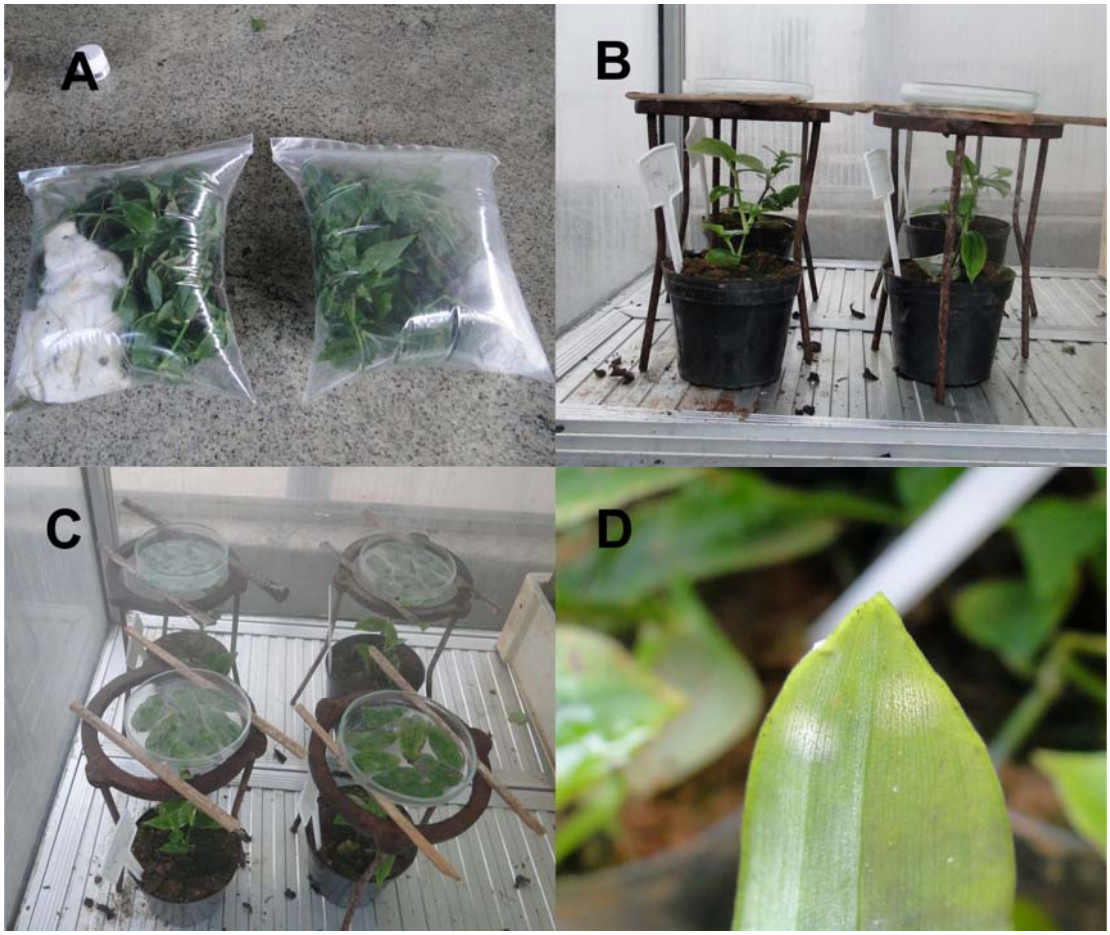

Fig 3. A. Two zip-lock bags containing stems of *T. fluminensis* bearing colonies of *K. brasiliensis* and humid paper tissue to simulate transportation conditions.. **B.**. Close-up of two apparatus for basidiospore discharge within dew chamber. **C**. View from above of four apparatus within dew chamber. **D.** Newly formed colonies of *K. brasiliensis* on a test-plant (treatment with incubation of two days within the plastic bag).