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Information package to support application to release the white smut-like fungus *Kordyana brasiliensis* for the biological control of wandering trad (*Tradescantia fluminensis*) in Australia

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Acknowledgments

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Executive summary

The nomination of wandering trad (*Tradescantia fluminensis*; Commelinaceae) as a target for biological control in Australia was approved in December 2015 by the Invasive Plants and Animals Committee (IPAC), a cross-jurisdictional sectoral sub-committee of the National Biosecurity Committee.

Surveys of natural enemies of wandering trad in Brazil, the region of origin of this weed, undertaken by Brazilian colleagues in 2005-09 and financially supported by Landcare Research, New Zealand, discovered a novel fungal species, the white smut-like fungus *Kordyana brasiliensis*. In a series of host-specificity tests performed on 20 non-target plant species in Brazil, the fungus was found to be highly specific. On the basis of these results it was approved in 2013 for release in New Zealand for the biological control of wandering trad. First releases in New Zealand are planned for November 2017.

Kordyana brasiliensis was imported into the BC3 Microbiological Area (AA A1280) of the CSIRO Black Mountain Containment Facility in Canberra in July 2014. Once a culture of the fungus was established on Australian accessions of wandering trad, a series of tests were performed to further investigate its host-range on non-target plant taxa. The selection of plant taxa for testing was based on recent molecular phylogenies of the family Commelinaceae. Tests were performed on 7 species that had previously been tested in Brazil (including the target weed) and 22 additional species or cultivars of relevance to Australia (ornamental, weed and native taxa). Taxa tested included representatives from the subtribe Tradescantiinae in the Tradescantieae tribe (to which wandering trad belongs) and representatives from across the other subtribes in the Tradescantieae tribe as well as from the other two tribes (Commelineae and Cartonemateae) in the Commelinaceae family in Australia. Each taxon was tested in two separate trials (five replicates per taxon per trial unless otherwise indicated) and wandering trad plants were used as positive controls.

Results obtained in the tests performed in Australia further demonstrated that *K. brasiliensis* is highly host specific. The fungus successfully developed and produced normal lesions only on the 14 Australian accessions of wandering trad tested. Only five taxa developed a limited number of small flecks, either water-soaked in appearance or necrotic, following inoculation with *K. brasiliensis* and were rated as resistant: *Aneilema acuminatum*, *Tradescantia* sp. Giant leaf (Browns Reserve accession), *Commelina* aff. *diffusa* (Northern Territory accession), *Pollia macrophylla*, *Pollia crispata*. All other non-target plant taxa tested did not develop any visible symptoms.

These results indicate that the level of risk associated with releasing *K. brasiliensis* in Australia is acceptable. Observations made during surveys in Brazil support that it will be a potentially effective biological control agent for wandering trad. Permission for its release in Australia is thus sought.

1 Information on target species in Australia

1.1 Taxonomy

Clade:	Commelinids, monocots
Order:	Commelinales
Family:	Commelinaceae
Tribe:	Tradescantieae
Genus:	*Tradescantia*
Species:	*fluminensis* Vell.
Common name:	Wandering trad, wandering tradescantia, wandering Jew, wandering creeper, water spiderwort
Synonyms:	*Tradescantia albiflora* Kunth

1.2 Description

Wandering trad is a long-lived perennial, prostrate, herb. It has fibrous roots and branched, hairless and somewhat succulent stems, which can produce roots at the nodes (Fig. 1A). Leaves are alternate, subsessile, glabrous, ovate-lanceolate, 2.5–5.5 cm long and 1–2.5 cm broad. Inflorescence consists of 15–20 white flowers each with three 7–10 mm long petals, 1–2 cm long pedicel and bearded stamina filaments (Walsh and Entwisle 1994) (Fig. 1B).

It is primarily spread via stem sections by water, soil movement and in garden waste. It is not known to set seed in Australia (Blood 2001, Global Invasive Species Database 2016).

1.3 Native range

Wandering trad is native to Southeast Brazil (Global Invasive Species Database 2016), but also found in neighbouring areas (Uruguay and Argentina) (CABI 2016).

1.4 Distribution

Wandering trad is most common and invasive in the coastal regions of NSW, Victoria and south-east Queensland (Fig. 2). It is also naturalised in South Australia, Western Australia, Tasmania, north Queensland and in some inland regions of Victoria. In frost-prone areas, its distribution is restricted to sheltered habitats.

Wandering trad is also widely naturalised overseas in: New Zealand, Europe (Portugal, Italy), Africa (South Africa, Swaziland, Kenya), Asia (Russia, Japan), Central and South America (Bermuda, Puerto Rico, Saint Lucia, Argentina) and USA (Hawaii, California, Florida, Georgia, Alabama, Louisiana, Kentucky, North Carolina, Texas) (The Global Invasive Species Database 2016).

Figure 1 A. Wandering trad (*Tradescantia fluminensis*): A. dense infestation in the Sydney region (photo: sydneyweeds.org.au), B. close-up of flowers.

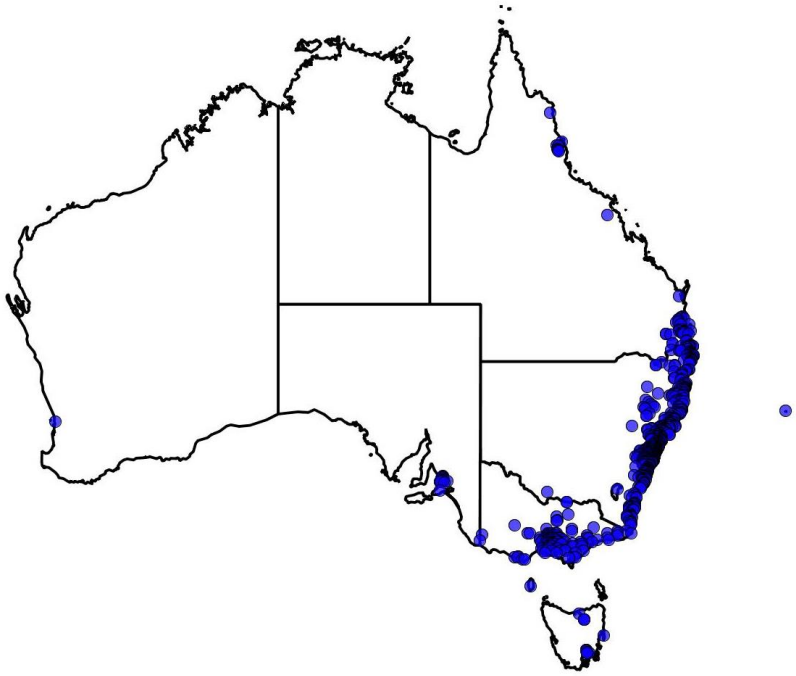

Figure 2 Current distribution of wandering trad (*Tradescantia fluminensis*) in Australia (Reproduced from Atlas of Living Australia 2016).

1.5 Summary of economic and environmental losses caused by the target

Wandering trad is a significant environmental weed in Australia (Dugdale et al. 2015). It typically requires disturbance, such as increased light and soil nitrogen to establish and become invasive in new areas. It forms dense mats up to 6 cm deep (Blood 2001) that smoothers vegetation and kills seedlings, leading to a

major decrease in species richness and abundance of native plants (Standish et al. 2001). Wandering trad mats can persist on the forest floor even after light gaps have closed. In addition to directly killing regenerating native seedlings, it can also increase the rate of decomposition, thus altering nutrient availability (e.g. higher available nitrogen) (Standish et al. 2004).

Wandering trad commonly causes skin allergy in dogs and can also cause allergic skin reactions in humans, although this is rare (Marsella et al. 1997, Paulsen and Thormann 2010).

1.6 Other control methods available

Manual removal of wandering trad is suitable for control of small infestations (Dugdale et al. 2015). Great care however, must be taken to remove all material since new plants can successfully establish from very small stem fragments.

Chemical control is considered more practical for the management of large infestations, although there is potential for non-target negative effects on native flora. The herbicides fluroxypyr and picloram are registered for use on wandering trad in Australia.

Standish (2002) found that herbicide applications and manual removal did not prevent re-growth of wandering trad after three successive treatments in New Zealand. Artificial shading was the most effective method for sustained control of wandering trad, without invasion by other weeds. It was acknowledged however, that artificial shading would be impractical for controlling large areas of wandering trad. Planting of native species into forest remnants infested by wandering trad to enhance natural vegetation cover was suggested as a possible option to achieve long-term control of the weed.

1.7 Information on all other relevant Commonwealth, State and Territory legislative controls of the target species

Although an important environmental weed in NSW and Victoria, wandering trad is not declared a noxious weed in these states under the *Noxious Weeds Act 1993* [1] and *Catchment and Land Protections Act 1994* [2], respectively. It is also not declared noxious in any other states and territories.

In Western Australia, it is included in the Permitted (S11) list[3] and therefore allowed entry into that state without an import permit.

1.8 Whether and when the target species was approved as a target species, and the proposing organisation

The nomination of wandering trad as a target for biological control in Australia was proposed by the Victorian Department of Economic Development, Jobs, Transport & Resources and approved in December 2015 by the Invasive Plants and Animals Committee (IPAC), a cross-jurisdictional sectoral sub-committee of the National Biosecurity Committee (Appendix A).[4]

[1] http://weeds.dpi.nsw.gov.au/Weeds/Details/141
[2] http://www.depi.vic.gov.au/agriculture-and-food/pests-diseases-and-weeds/protecting-victoria-from-pest-animals-and-weeds/legislation-policy-and-permits/new-noxious-weed-and-pest-animal-declarations
[3] https://www.agric.wa.gov.au/organisms?search_string=Tradescantia+fluminensis+Vell.
[4] For more information on process see http://www.weeds.org.au/target.htm

2 Information on biological control agent

2.1 Agent name

Class: Exobasidiomycetes

Order: Exobasidiales

Family: Brachybasidiaceae

Genus: *Kordyana* Racib.

Species: *brasiliensis* D.M. Macedo, O.L. Pereira & R.W. Barreto sp. nov.

Common Name: Wandering trad white smut-like fungus

Voucher: Minas Gerais, Viçosa, 10 December 2009, D. M. Macedo (VIC 31367) – HOLOTYPE: lodged with the herbarium at the Universidade Federal de Viçosa (Herbarium VIC)

Voucher specimen: A voucher herbarium specimen will be deposited in the Plant Pathology Herbaria of NSW Department of Agriculture, Orange, and of the Queensland Department of Agriculture and Fisheries, Brisbane, as soon as permission is granted to release the fungus in Australia.

2.2 Brief biology of the agent

An undescribed *Kordyana* sp. was found on *T. fluminensis* during surveys in Brazil (Pereira et al. 2008). Based on morphological and molecular characteristics, it was recently described as a new species, *Kordyana brasiliensis* (Macedo et al. 2016). This fungus has not been found on any other species in Brazil.

Kordyana brasiliensis produces single-celled, hyaline basidiospores that are oblong to elliptic, 3–5 × 8–15 µm, and smooth-walled. The basidiospores form on basidia, which emerge through the stomata of infected host plants (Barreto et al. 2010; Macedo et al. 2016). Each basidium bear two sterigmata, each producing a basidiospore that is forcibly ejected.

Basidiospores germinate readily on plant tissue, providing some moisture is present, and penetrate via stomata. First visible signs of infection are observed on the under surface of leaves 7 to 8 days after inoculation. A few days later, lesions first appear on upper surface of leaves as diffuse chlorotic spots, which become more prominent as chlorosis spreads (Fig. 3A). Within 15 days of inoculation, whitish lesions developed on the under surface of leaves begin producing basidiospores if exposed to high humidity (Fig. 3B). The central area of lesions on the upper leaf surface turns reddish brown as they mature, eventually becoming necrotic (Fig. 3C). Coalescing lesions lead to complete necrosis and death of leaves (Fig. 3D).

2.3 Native range of the agent

Kordyana brasiliensis has only been recorded from Brazil. It was found on *T. fluminensis* in the following Brazilian states during surveys performed over a 5-year period: Minas Gerais, Paraná, Santa Catarina, São Paulo and Rio Grande do Sul (Pereira et al. 2008, Barreto et al. 2010).

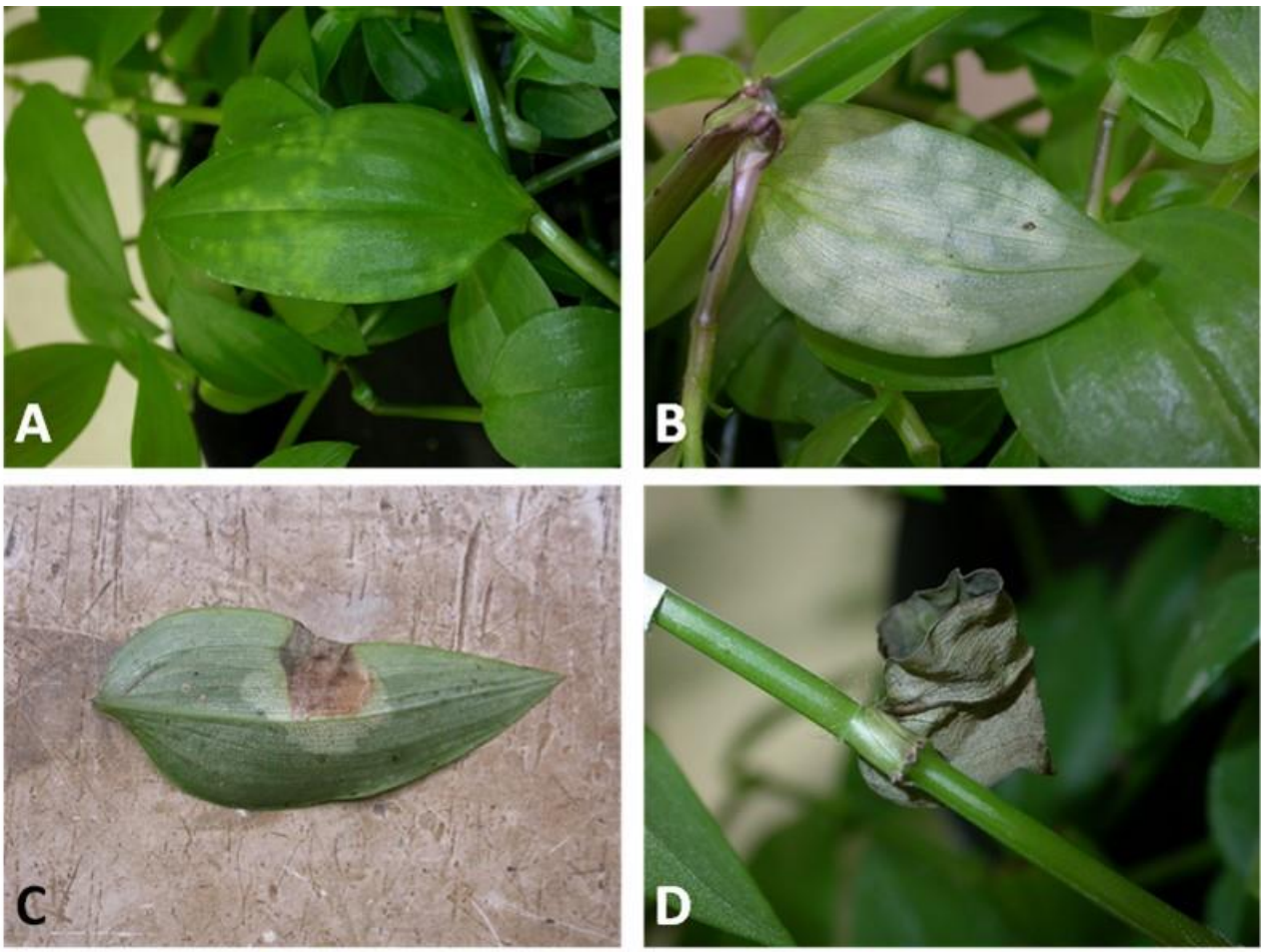

Figure 3 Disease symptoms caused by *Kordyana brasiliensis* on leaves of *Tradescantia fluminensis*. Diffuse chlorotic spots on the upper surface of leaves (A) and corresponding whitish lesions on the under surface of leaves (B) at 14 days after inoculation. Lesions become necrotic as they mature (C), eventually causing complete necrosis and death of leaves (D).

2.4 Related species to the agent and a summary of their host range

The distinct morphological features of *Kordyana brasiliensis* and a phylogenetic analysis of large subunit (LSU) ribosomal RNA gene sequences strongly support that it represents a new species, which is different to *Kordyana tradescantiae* (syn. *Exobasidium tradescantiae*) (Barreto et al. 2010, Macedo et al. 2016).

The fungus *K. tradescantiae* has been recorded in Central and South America on *Tradescantia* spp. and a few other plant species within the family Commelinaceae: *Tradescantia* sp. (Costa Rica, Ecuador), *Tradescantia poelliae* (Costa Rica, Panama), *Aneilema* sp. (Ecuador), *Aneilema ovato-oblongum* (Ecuador), *Callisia umbellulata* (Ecuador) (Petrak 1950, Gómez and Kisimova-Horovitz 1997, Piepenbring et al. 2010, Farr et al. 2015). It has also been recorded on *Tradescantia ohiensis* in Florida, USA (Leahy 2009 cited in Piepenbring et al. 2010). *Kordyana tradescantiae* was described in 1900 from *Tradescantia capitata* in Java, Indonesia (Leahy 2009 cited in Piepenbring et al. 2010, Farr et al. 2015).

Only nine species of *Kordyana* are listed in the Global Biodiversity Information Facility (GBIF) (2013) (Table 1).

Table 1 Species of *Kordyana* listed in the Global Biodiversity Information Facility [A] (2013), with their respective recorded plant hosts according to Farr et al. (2016) (unless indicated otherwise).

KORDYANA SPECIES	PLANT HOSTS
K. aneilemae	*Aneilema angustifolium*
K. boswelliae	*Boswellia* sp.[B]
K. celebensis	*Commelina benghalensis*, *Commelina diffusa* [C], *Commelina ensifolia* [D]
K. commelinae	*Commelina communis, Commelina nudiflora*
K. cyphelloidis	Species in the family Bignoniaceae [B]
K. indica	*Commelina benghalensis* [B]
K. polliae	*Commelina maculata* [B], *Pollia japonica*, *Pollia sorzogonensis* [B]
K. polliniae	*Microstegium japonicum* [B, E]
K. tradescantiae	*Tradescantia* sp., *Tradescantia capitata*, *Tradescantia ohiensis* [F], *Tradescantia poelliae*, *Aneilema* sp., *Aneilema ovato-oblongum*, *Callisia umbellulata*

[A] http://www.gbif.org/species
[B] According to Index Fungorum (http://www.indexfungorum.org/).
[C] CABI (2016).
[D] Plant Health Australia (2001).
[E] Currently accepted name for *Pollinia japonica* according to The Plant List (http://www.theplantlist.org/)
[F] Leahy 2009 cited in Piepenbring et al. 2010.

2.5 Proposed source(s) of the agent

The accession of *K. brasiliensis* used in all host-specificity tests performed in the BC3 Microbiological Area of the CSIRO Black Mountain Containment Facility in Canberra (AA A1280) (Import permit no. IP4008334) originated from the ground of the Universidade Federal de Viçosa, MG, Brazil. It was imported in quarantine in July 2014. This is the same accession that was used in testing carried out in Brazil as part of the New Zealand biological control program on wandering trad. The fungus was approved in 2013 for release in New Zealand[5] and the first releases occurred in March 2018 (Landcare Research NZ staff, pers. comm.).

2.6 Agent's potential for control of the target

Kordyana brasiliensis is very similar to the fungus *Entyloma ageratinae* that has been highly successful as a biological control agent of mistflower (*Ageratina riparia*) in Hawaii, New Zealand and more recently Australia (Trujillo 2005, Barton et al. 2007, Morin et al. 2012). Both fungi belong to the same class (Exobasidiomycetidae) and have similar life cycles.

Like other *Kordyana* spp., *K. brasiliensis* establishes a close interaction with the living cells of its host leaves, rather than killing the host cells as part of the infection process. These interaction sites are located in intercellular hyphae attached to host cells (Begerow et al. 2002). Through this continuous absorption or diversion of assimilates from the plant, the fungus is detrimental to plant development. The fungus also cause extensive necrosis on leaves, destroying leaf tissue and thus reducing the photosynthetic surface of the plant.

[5] www.epa.govt.nz/Documents/APP201362_decision.pdf

2.7 Information on non-target organisms at risk from the agent

Kordyana brasiliensis is a novel species, not recorded before on *T. fluminensis* or on any other species in the family Commelinaceae or in other plant families (Macedo et al. 2016). Other *Kordyana* spp. however, have been recorded from a range of plant species within the family Commelinaceae (Table 1).

In Australia, there are no crop plants in the family Commelinaceae (Appendix B). There are many species within this family that have been introduced to Australia for horticultural purposes, some of which now recorded as naturalised. There are a number of Australian native species within the family. Only one of these species (*Cyanotis axillaris*) belongs to the same tribe (Tradescantieae) as wandering trad.

2.8 Possible interactions, including conflict-of-interest with existing biological control programs

No biological control agents have been released for wandering trad in Australia.

Additional host-specificity testing on one of the three insect biological control agents (chrysomelid beetles) released in New Zealand between 2011–13 (Fowler et al. 2013), the tradescantia leaf beetle *Neolema ogloblini*, is currently being carried out at the AgriBio quarantine facility in La Trobe, Victoria by collaborators from the Department of Economic Development, Jobs, Transport & Resources. If it is deemed not to pose a risk to non-target plants in Australia, an application for its release will be submitted to the authorities.

Damage caused by these three beetle species to leaves, shoot-tips and mature stems of *T. fluminensis* in the native range (Brazil) is impressive. All three beetle species are now confirmed as established in New Zealand and their populations are building-up (Landcare Research NZ staff, pers. comm.). Initial damage caused by the beetle species is encouraging and their impact in suppressing the weed will continue to be closely monitored in New Zealand. Leaf infection by *K. brasiliensis* is expected to complement beetles damage when it is released in New Zealand.

2.9 Information on where, when and how initial releases will be made

Upon obtaining approval to release *K. brasiliensis* in Australia, wandering trad leaves with lesions caused by *K. brasiliensis* will be placed on water agar contained in Petri dishes and removed from the quarantine facility (in the presence of relevant officers from the Department of Agriculture and Water Resources). A large number of wandering trad plants, grown and maintained in the CSIRO glasshouses at Black Mountain, Canberra, will then be inoculated using these infected leaves in a controlled-environment room. Wandering trad plants with *K. brasiliensis* disease symptoms will be used to establish infections in the field at selected sites across the weed's range (mainly in Victoria and NSW). Disease development and spread will be closely monitored during the first growing season.

Redistribution of *K. brasiliensis* from infected to non-infected sites may be necessary since basidiospores are fragile and not known to travel long distances on wind currents.

2.10 Host-specificity of the agent

2.10.1 HOST-SPECIFICITY TESTING UNDERTAKEN IN BRAZIL

Kordyana brasiliensis was tested on a range of species within and outside the family Commelinaceae by the Brazilian research team who discovered and described the fungus. These researchers were contracted by Landcare Research New Zealand to undertake host-specificity testing as part of their biological control program for wandering trad (Barreto and Macedo 2011, Fowler et al. 2013). The methodology and results of these tests are supplied in Appendix C. On the basis of these results[6], the fungus was approved in 2013 for release in New Zealand[7] and the first releases occurred in March 2018 (Landcare Research NZ staff, pers. comm.).

Test plant species for the host-specificity tests performed in Brazil were selected phylogenetically, and included introduced species in New Zealand that belong to the same genus, sub-tribe, tribe and family as the target weed (Table 2). It is noteworthy that there are no plant species native to New Zealand that belong to the Commelinaceae family. Additional species tested belonged to other families related to the Commelinaceae (same order and same class).

2.10.2 HOST-SPECIFICITY TESTING UNDERTAKEN IN A CONTAINMENT FACILITY IN AUSTRALIA

Test list

Six of the non-target species tested in Brazil were included in the tests performed in the CSIRO Black Mountain Containment to provide confidence in results obtained in previous testing. The tests included a wide range of native and ornamental species within the Commelinaceae that occur in Australia (Table 2). The test list was compiled according to the phylogenetic centrifugal approach of Wapshere (1974), which places greater representation on the more closely related species to the target weed. No unrelated crop species were included in the test list since these species do not contribute to the delineation of the host range of specialised biological control agents (Briese 2003, Sheppard et al. 2005).

Recent published papers on the molecular phylogeny of Commelinaceae (Evans et al. 2003, Wade et al. 2006, Burns et al. 2011) were used to devise the test list so that species most closely related to wandering trad that are present in Australia were given priority (Appendix B). The test list comprised several representatives from the Tradescantieae tribe, the tribe that wandering trad belongs to, including the only native species of this tribe in Australia, *Cyanotis axillaris* (Table 2). The list also included representative species in other tribes related to Tradescantieae within the subfamily Commelinoideae. One species in the tribe Cartonemateae that belongs to the other subfamily within Commelinaceae (Cartonematoideae) was also included in the testing.

A representative species from the family Pontederiaceae, which is in the same order (Commelinales) of the family Commelinaceae, was included in tests in Brazil and found not to support development of the fungus (Table 2, Appendix C). On that basis and in light of the lack of development of the fungus on species in the Commelinaceae tested in Brazil, except the target weed, it was not deemed necessary to include representatives of other families in the order Commelinales (i.e. Haemodoraceae, Hanguanaceae and Philydraceae). Representatives from families in different orders but in the same class as Commelinaceae, including two species in the Zingiberaceae, were also tested in Brazil and did not develop any disease symptoms (Table 2, Appendix C).

[6] http://www.epa.govt.nz/Documents/APP201362_application.pdf
[7] www.epa.govt.nz/Documents/APP201362_decision.pdf

Table 2 List of non-target plant taxa used to test the specificity of *Kordyana brasiliensis* in Brazil (as part of the New Zealand biological control program for wandering trad) and in a containment facility in Australia. Summary results from host-specificity tests are presented.

ORDER	FAMILY	SUBFAMILY	TRIBE	SUBTRIBE	RELATIONSHIP TO TARGET WEED		PLANT TAXON [1]	DISEASE SYMPTOMS [2]	
								BRAZIL TESTS	AUSTRALIA TESTS
Commelinales	Commelinaceae	Commelinoideae	Tradescantieae	Tradescantiinae	Target weed	1	*Tradescantia fluminensis*	yes	yes
					Same genus	2	*Tradescantia* sp. Giant leaf [3]	-	no
						3	*Tradescantia pallida*	no	no
						4	*Tradescantia spathacea*	no	no
						5	*Tradescantia zebrina*	no	no
						6	*Tradescantia zononia*	no	-
						7	Tradescantia 'Cindy' [4]	-	no
						8	Tradescantia 'Isis' [4]	-	no
						9	Tradescantia 'J C Wegellin' [4]	-	no
						10	Tradescantia 'Nutshell Rosy' [4]	-	no
						11	Tradescantia 'Snowflake' [4]	-	no
						12	Tradescantia 'Sweet Kate' [4,5]	-	no
					Same sub-tribe	13	*Callisia repens*	no	no
						14	*Callisia warszewicziana*	no	-
						15	*Gibasis geniculata*	-	no
						16	*Gibasis schiediana*	no	-
						17	*Tripogandra diuretica*	no	-
				Cyanotinae	Same tribe	18	*Cyanotis axillaris* [6]	-	no
				Dichorisandrinae		19	*Dichorisandra thyrsiflora*	no	no
						20	*Siderasis fuscata*	no	-
				Thyrsanthemineae		21	*Tinantia* sp.	no	-
			Commelineae		Same subfamily	22	*Aneilema acuminatum* [6,7]	-	no
						23	*Aneilema biflorum* [6]	-	no
						24	*Commelina benghalensis*	no	-

					25	*Commelina ciliata* [6]	-	no
					26	*Commelina cyanea* [6]	-	no
					27	*Commelina diffusa* [6,8]	no	no
					28	*Commelina* aff. *diffusa* [6,9]	-	no
					29	*Commelina ensifolia* [6]	-	no
					30	*Commelina erecta*	no	-
					31	*Commelina lanceolata* [6]	-	no
					32	*Floscopa scandens* [6]	-	no
					33	*Murdannia gigantea* [6]	-	no
					34	*Murdannia graminea* [6,7]	-	no
					35	*Pollia crispata* [6]	-	no
					36	*Pollia macrophylla* [6]	-	no
		Cartonematoideae	Cartonemateae	Same family	37	*Cartonema philydroides* [6]	-	no
	Pontederiaceae			Same order	38	*Eichhornia crassipes*	no	-
Poales	Cyperaceae			Same class	39	*Cyperus rotundus*	no	-
	Juncaceae				40	*Juncus* sp.	no	-
Zingiberales	Strelitziaceae				41	*Strelitzia reginae*	no	-
	Zingiberaceae				42	*Hedychium coronarium*	no	-
					43	*Zingiber officinale*	no	-

[1] Identification of species tested in Australia was confirmed by expert taxonomist at The University of Adelaide.
[2] Disease symptoms are in the form of yellow lesions on the upper surface of leaves with corresponding whitish lesions on the under surface from which spores of the fungus are produced, thus completing the life cycle. See Figure 3.
[3] Collected along creek at Browns Reserve, Greensborough, 3088, Victoria (Access off Albion Cres) (-37.692311°, 145.122125°). Taxonomist from The University of Adelaide determined that it is not *T. fluminensis* as we know it and possibly a different species, not previously reported in Australia. However, it is not possible at this stage to attribute it to an existing species because there is no recent and robust South American account of the genus. Consequently, it is formally referred to the phrase name *Tradescantia* sp. Giant leaf.
[4] Cultivars used as ornamentals in Australia.
[5] *Tradescantia ohiensis* X (*subaspera* X *virginiana*) (Anderson Group). Andersoniana Group include biflorums hybrids of *T. virginiana*, *T. subaspera* and *T. ohiensis*.
[6] Native to Australia.
[7] A different accession of this taxon was used in each trial, based on availability of plant material at the time of testing (Appendix D).
[8] This native Australian species is present in cultivation in New Zealand and hence was included in the testing performed in Brazil.
[9] Expert taxonomist from The University of Adelaide was not absolutely certain of the identification of this species from the Northern Territory, but believed it was closest to *C. diffusa*, hence the name *Commelina* aff. *diffusa* (aff. is short for "affinis" meaning "similar to", which indicates that the identification is not certain).

During the process to source various accessions of wandering trad for testing, we obtained plants with large leaves from Browns Reserve, Dandenong, Victoria that were originally thought to be *T. fluminensis*. The expert taxonomist on Commelinaceae, from The University of Adelaide, consulted determined that the Browns Reserve accession is not *T. fluminensis* as we know it and possibly a different species, not previously reported in Australia. However, he stated that it is not possible at this stage to attribute it to an existing species because there is no recent and robust South American account of the genus. Consequently, it is formally referred to the phrase name '*Tradescantia* sp. Giant leaf'.

Materials and methods

Test plants

Wandering trad plants from the accession used as control in all tests (Kangaroo Valley accession; -34.6867°, 150.6186°) as well as 13 other Australian accessions of wandering trad (Appendix D) were propagated using stem cuttings. Cuttings were planted into potting mixture (5:1:1:3 straw-based compost, peat moss, river sand, perlite, with 1.4 kg slow-release fertilizer m^{-3} [Aboska®, N:P:K 15.16:6.93:5.19]) and placed in a glasshouse (16–26°C; natural light). Plants were fertilised fortnightly with liquid fertiliser (Aquasol™; N=23, P=4, K=18).

The various non-target plant taxa to be tested (Table 2) were propagated from cuttings from field plants, or obtained as whole plants from the field or nurseries (Appendix E), and grown in the glasshouse (conditions as above; if required, additional lighting with metal halide lights was provided to maintain a 12-h photoperiod). Actively growing plants were taken into the BC3 area of the CSIRO Black Mountain Containment Facility for testing.

Production of *K. brasiliensis* inoculum

Every week, three wandering trad plants (Kangaroo Valley accession) with abundant foliage (in 10-15 cm diam. pots) were inoculated with *K. brasiliensis* to maintain a continuous supply of inoculum for host-specificity tests. Leaves with several lesions were excised from infected wandering trad plants (approx. 4 wks after inoculation) and each deposited (upper surface down) onto the slightly melted surface of a 2% water agar block (approx. 1 cm^2) placed in the base of a 15 cm diam plastic Petri dish (five blocks per dish). Each dish containing infected leaves (without lids) was then fixed with sticky tape to the inside bottom of a 25 L opaque plastic bucket. Each bucket with infected leaves was inverted over the opening of another 25 L bucket containing one wandering trad plant misted with water. Special care was taken to arrange the foliage to ensure that the under surface of several leaves faced upward. In preliminary work, it was discovered that *K. brasiliensis* infects wandering trad by producing germ-tubes that enter leaves via stomata, which are located on the under surface of leaves. The double-bucket inoculation chambers were placed in a controlled-environment room at 20°C for 48 h. During that period, lesions produced abundant basidiospores that were naturally discharged onto the plant foliage. Plants were then removed from the buckets and placed on the bench of the controlled-environment room (12 h photoperiod, fluorescent lights).

Susceptibility of Australian accessions of wandering trad

The susceptibility of all Australian wandering trad accessions, sourced and grown in the glasshouse (Appendix D), to *K. brasiliensis* were determined in a single large trial comprising two replicate plants per accession. *Tradescantia* sp. Giant leaf (Browns Reserve accession) (Table 2) was also included in the trial because it was thought to be a large-leaved biotype of *T. fluminensis* at the time of this trial.

Three agar blocks, each with a wandering trad leaf with several lesions of *K. brasiliensis* (as described above in section 'Production of *K. brasiliensis* inoculum'), were placed in the base of a 9 cm diam Petri dish. Each dish with infected leaves (without a lid) was fixed with sticky tape to the inside bottom of a 10 L opaque plastic bucket, which was then inverted over the opening of another 10 L bucket that contained one plant (with foliage arranged so that the under surface of several leaves faced upward), which had been misted with water. The double-bucket inoculation chambers were placed in a controlled-environment room

(conditions as above). After 48 h, each inoculation set-up was dismantled and the plant was removed from the bucket and placed on the bench of the controlled-environment room. At 14 and 28 days after the inoculation period, all leaves on each plant were examined for visible disease symptoms.

Host-specificity tests

Each plant taxon (including *Tradescantia* sp. Giant leaf) was tested in two separate trials to account for any possible variation in disease development across time (Table 2). Healthy plants (up to 30 cm in height including pot) were chosen for each trial (five plant replicates per taxon per trial unless indicated otherwise). Nine trials consisting of up to eight taxa each and including the positive control wandering trad were performed.

The host-specificity tests were conducted under optimum conditions for infection of plants and disease development. Test plants were exposed to infected leaves of wandering trad with several well-developed lesions for 48 hrs in high humid conditions. This period provided more than sufficient time for the fungus to sporulate on leaf lesions and for spores to land, germinate and produce hyphae on the leaf surface of test plants, and for surface hyphae to penetrate through stomata and produce initial internal hyphae. Indeed, in the plant pathology literature plants inoculated with spores of foliar fungal pathogens are typically placed in high humid conditions overnight and infrequently for up to 24 hrs. We selected a 48 hrs incubation period in high humid conditions to ensure that a very high spore load landed on plants and the fungus had plenty of time to grow and infect. Available moisture is crucial in the initial infection phase of a fungal pathogen, i.e. until the fungus has penetrated the plant tissue and produced internal hyphae. Subsequent growth within the plant tissue and production of lesions are independent of ambient moisture conditions, because the fungus is obtaining nutrients and moisture from host cells.

Inoculated test plants were examined for visible disease symptoms at 28 days after inoculation – which is twice the period that it takes for lesions development on the host wandering trad (Fig. 3).

- Stage 1

The first stage of each trial involved inoculation of single leaves of test and control plants. One agar block with a wandering trad leaf bearing several lesions of *K. brasiliensis* (as described above in section 'Production of *K. brasiliensis* inoculum') was placed in the base of a 5 cm diam Petri dish. The dish with the infected leaf (without a lid), was attached to a fine bamboo stick with a metal clip and then inverted above the under surface of a single leaf or group of leaves (when very small) of one plant of each test and control taxon (Fig. 4). Two single leaves were inoculated with different dishes in one of the replicate plants of each taxon. Previous microscopic examinations of leaves of each test taxon revealed that stomata (which are the entry points for infection by *K. brasiliensis*) were either solely located or most abundant on the under surface of leaves of all taxa (as is the norm in many plant species). Narrow strips of masking tape were used to ensure that the under surface of the leaf to be inoculated was facing upward and that the dish with the infected wandering trad leaf remained lined up with the other leaf for the duration of the inoculation. For large or long leaves, the area to be inoculated was delineated with a black marker pen to facilitate subsequent observations for symptoms. The plant was then placed in a 10 L opaque plastic bucket, misted with distilled water, covered with another 10 L bucket and placed in a controlled-environment room (conditions as above). After 48 h, the inoculation set-up was dismantled and the plant was removed from the bucket and placed on the bench of the controlled-environment room. At 28 days after the inoculation period, each inoculated leaf was examined for visible symptoms of infection.

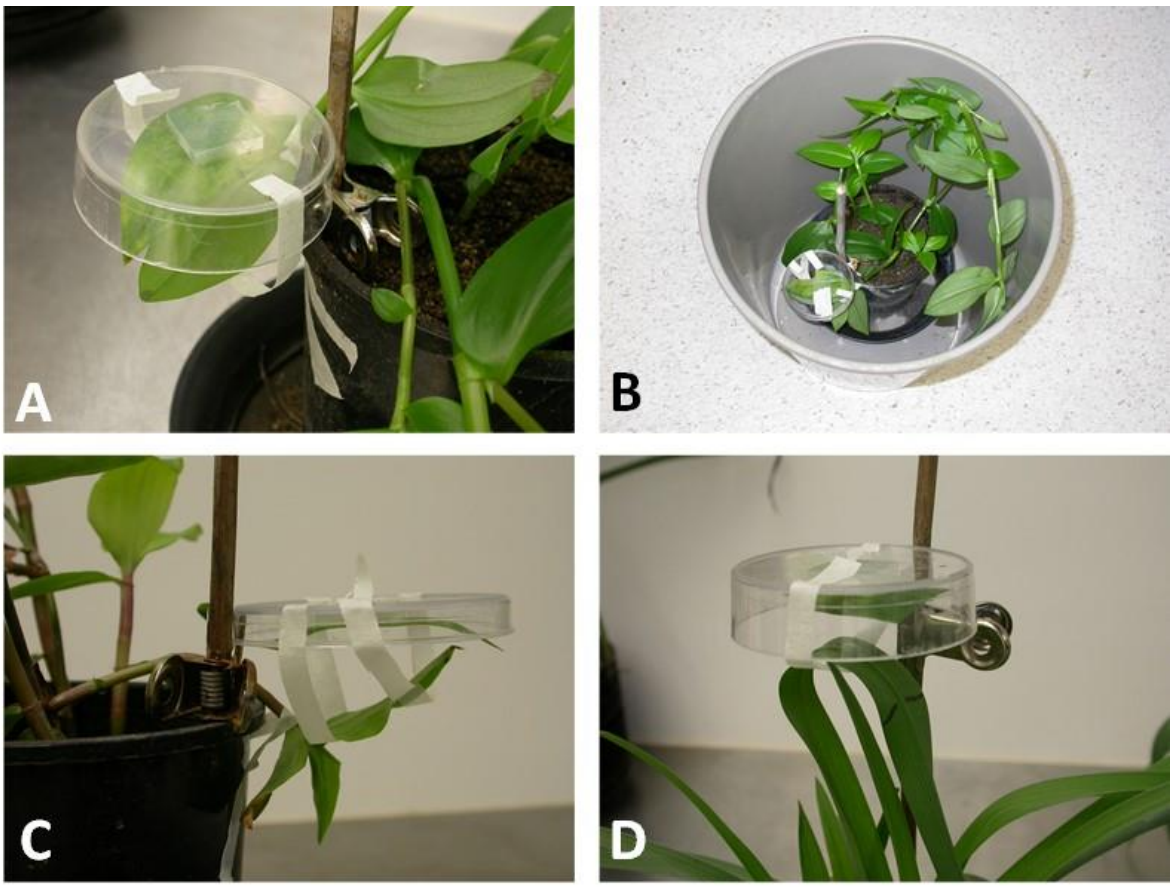

Figure 4 Experimental set up for inoculation of a single leaf with an infected wandering trad (*Tradescantia fluminensis*) leaf with several lesions of *Kordyana brasiliensis*. Inoculation of wandering trad (A & B), *Floroscopa scandens* (C) and Tradescantia 'Isis' (D) plants.

Microscopic examinations: One of the two leaves or groups of small leaves inoculated in one of the replicate plants of each taxon was excised 5 days after the beginning of the inoculation period and cut into small pieces (0.5–1 cm^2). The 5 days period was selected because preliminary work on wandering trad showed that by that time the fungus has penetrated the leaf tissue and developed an extensive network of intercellular hyphae. The pieces were cleared and stained in a solution containing aniline blue, ethanol, chloroform, lactic acid, phenol and chloral hydrate for 48 h (Bruzzese and Hasan 1983). They were then rinsed in water, placed in a saturated solution of chloral hydrate for 1 day and transferred back to water for storage. Prior to microscopic examination the pieces were placed in blue-lacto-glycerol stain on a microscope glass slide for 3–5 min. Excess stain was then gently removed with blotting paper and pieces were mounted in water and examined under a light microscope. At least 100 basidiospores per species were examined.

For taxa that developed visible symptoms, one of the inoculated leaf or group of small leaves with symptoms from one of the replicate plants was excised at 28 days after the inoculation period and processed as described above for microscopic examinations.

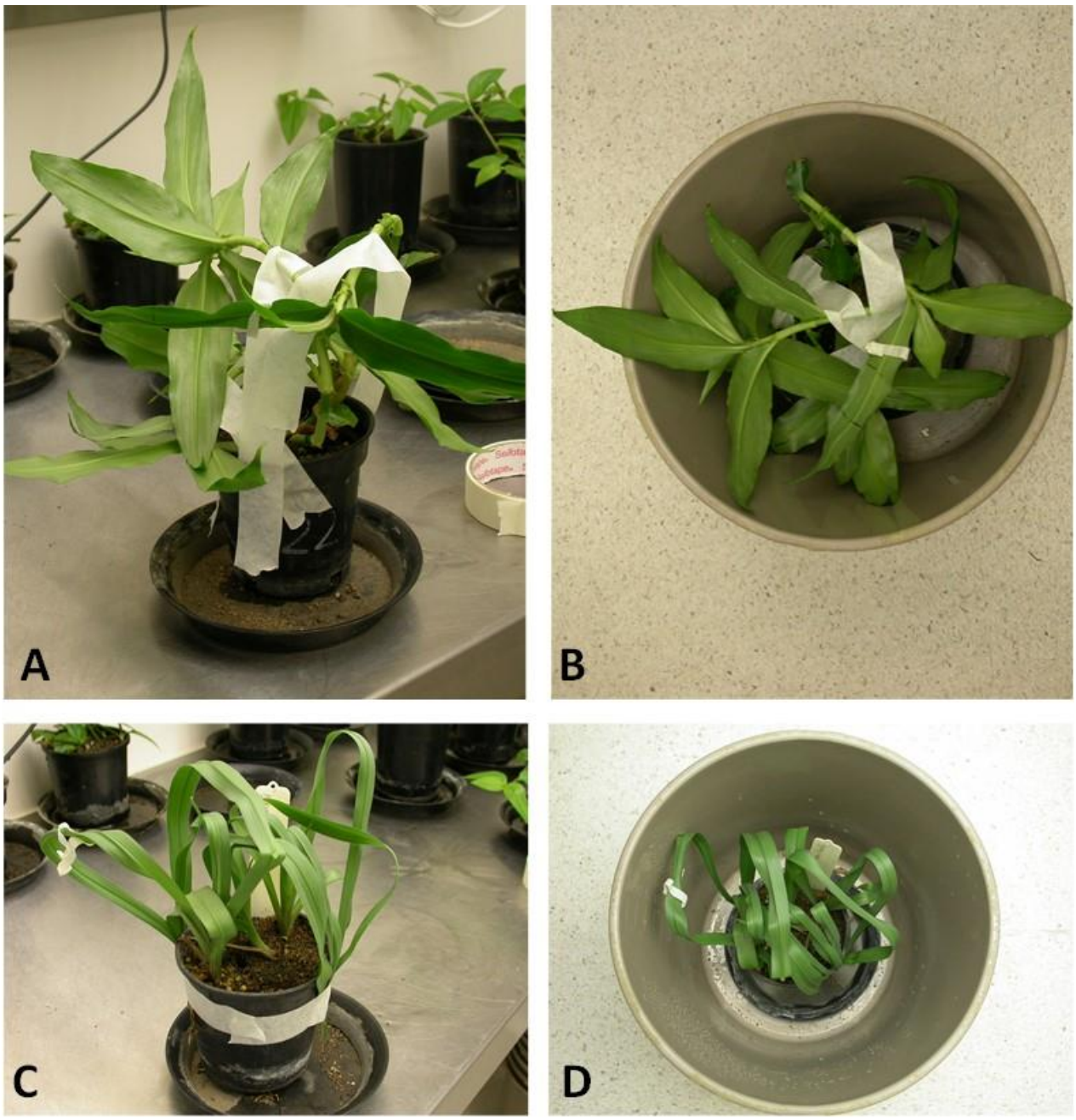

Figure 5 Experimental set up for inoculation of whole plants with *Kordyana brasiliensis*. Strips of masking tape or fine wire were used to ensure that the under surface of leaves was facing upwards prior to the exposure to wandering trad (*Tradescantia fluminensis*) leaves bearing several lesions of *Kordyana brasiliensis*. A & B *Pollia crispata*, C & D Tradescantia 'Snowflake'.

- Stage 2

The same plants used in Stage 1 were inoculated again using a whole-plant approach after their single inoculated leaves were examined for visible symptoms. The whole-plant inoculation approach was the same as that used in the trial to assess the susceptibility of Australian wandering trad accessions (see above). For some of the test plant taxa, masking tape or fine wire was used if necessary to ensure that the under surface of several leaves faced upward (Fig. 5). All leaves were examined for visible disease symptoms of infection at 28 days after the inoculation period.

Assessment of *Kordyana brasiliensis* development

The microscospic development of *K. brasiliensis* on leaves of test plants and subsequent visible symptoms were assessed according to 20 categories (Fig. 6). The susceptibility of the test plant species to the fungus was then classified according to visible symptoms observed using categories inspired from previously devised systems (Mortensen 1985, Bruzzese and Hasan 1986, Evans and Tomley 1994) (Table 3).

Results

All Australian wandering trad accessions tested were equally susceptible to *K. brasiliensis* and each developed a large number of normal lesions. *Tradescantia* sp. Giant leaf (Browns Reserve accession), which was originally thought to be *T. fluminensis* and thus included in the trial, did not develop any lesions and was thus included in subsequent host-specificity testing.

The full range of developmental stages of *K. brasiliensis* observed on the control wandering trad plants and on each of the test plant species is presented in Table 4. A summary of results, combined with previous results obtained in tests performed in Brazil, is presented in Table 2.

Microscopic development of *Kordyana brasiliensis* on leaf surface of tested taxa

Microscopic examinations of young leaves of wandering trad at 5 days after the beginning of the inoculation period with *K. brasiliensis* revealed extensive basidiospore germination, development of surface hyphae and penetration through stomata (Table 4, Fig. 7A, B, C). Networks of intercellular hyphae (Figs 7D, 8A), with several interaction sites between intercellular hyphae and host cells (Fig. 8B) were observed within the leaves. Well-developed sori with a basidial layer, the precursor to basidia on which basidiospores are produced, were present in stomatal chambers (Fig. 8C, D).

Germinated basidiospores and surface hyphae were observed on all other species (Table 4, Fig. 9A, B). The presence of penetration hyphae in stomata was detected in only some species: *A. acuminatum*, *A. biflorum*, *C.* aff. *diffusa* (NT accession), *G. geniculata*, *P. crispata*, *P. macrophylla*, and *Tradescantia* sp. Giant leaf (Table 4). Limited or necrotic/collapsed intercellular hyphae within the leaf tissue underneath stomata were observed in all of these species (Fig. 9C, D), except in *A. biflorum* where no intercellular hyphae were detected.

Development of visible symptoms of *Kordyana brasiliensis* on tested taxa

Only wandering trad developed abundant, normal and large lesions on leaves across the trials and consequently was rated as highly susceptible to *K. brasiliensis* (Table 4, Fig. 8E, F). When exposed to high humidity, basidiospores were produced on basidia that protrude through stomata on the under surface of leaves, giving lesions a ‘woolly white’ appearance (Figs. 3D, 8F). Lesions coalesced and became greyish-brown as the disease progressed (Fig. 3C), and entire leaves eventually died (Fig. 3D).

No other species developed normal disease symptoms of *K. brasiliensis*. The following species however, developed small flecks, either water-soaked in appearance or necrotic, following single-leaf or whole-plant inoculations and were rated as resistant (Table 4): *A. acuminatum*, *C.* aff. *diffusa* (NT accession), *P. crispata*, *P. macrophylla*, and *Tradescantia* sp. Giant leaf (Browns Reserve accession) (Fig 10). All other taxa tested did not develop any visible symptoms.

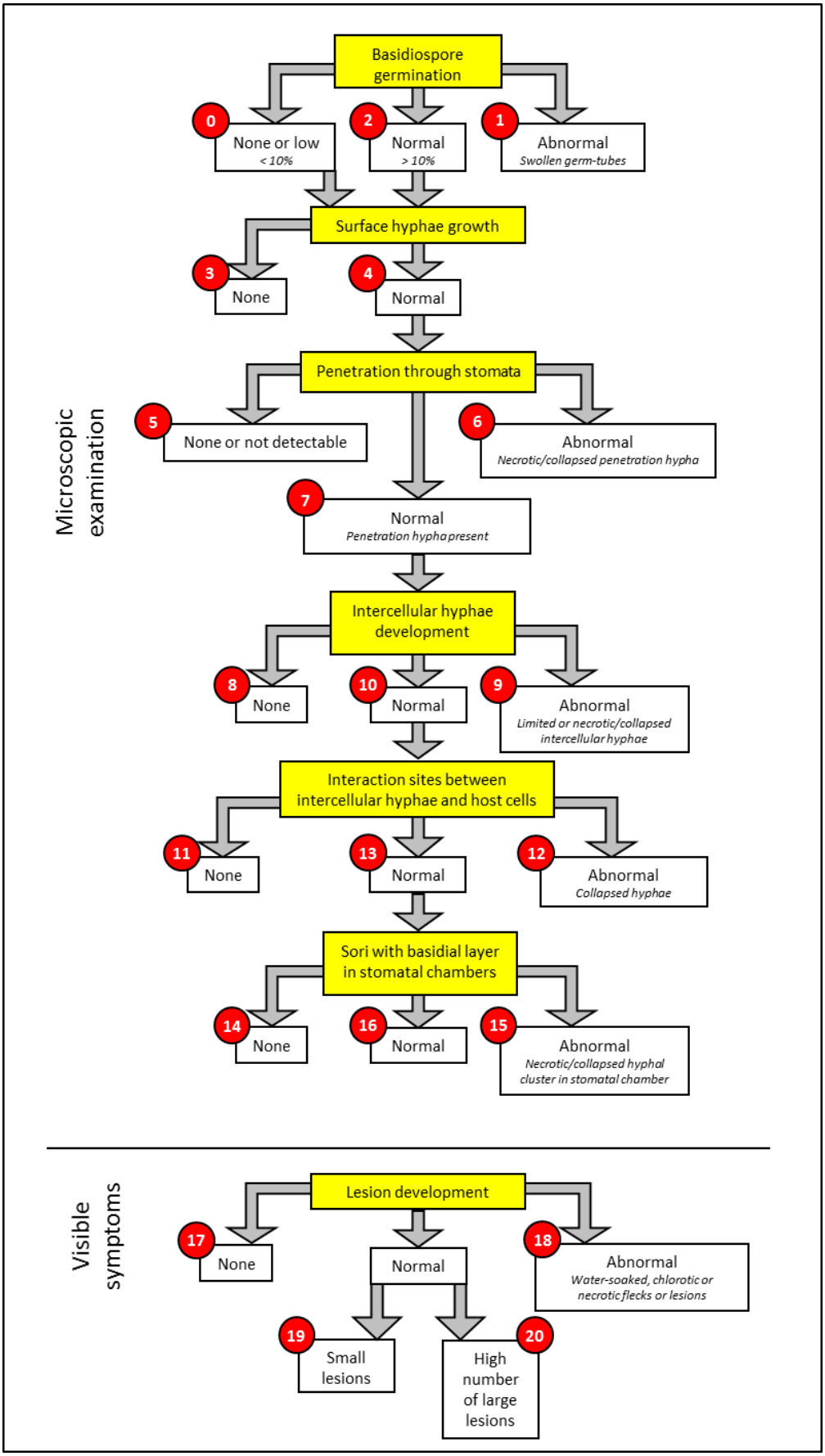

Figure 6 Schematic representation of the categories used to assess the microscopic development of *Kordyana brasiliensis* and visible symptoms on the test plant taxa in tests conducted in Australia.

Table 3 Categories used to classify the susceptibility of test plant taxa to *Kordyana brasiliensis* in tests conducted in Australia.

CATEGORIES	VISIBLE SYMPTOMS	DEVELOPMENTAL STAGE OF FUNGUS
Immune (I)	None	No sign of penetration
Highly resistant (HR)	None	Some penetration with no or abnormal intercellular hyphae development.
Resistant (R)	Water-soaked, chlorotic or necrotic flecks or spots present.	Some penetration and abnormal/limited intercellular hyphae development. Plant host cell necrosis present.
Susceptible (S)	Normal lesions developed but restricted in size.	Network of intercellular hyphae present with interaction sites between hyphae and host cells observed. Sori with basidial layer present in stomatal chambers.
Highly susceptible (HS)	High numbers of normal, large lesions present.	Network of intercellular hyphae present with interaction sites between hyphae and host cells observed. Sori with basidial layer present in stomatal chambers.

Table 4 Microscopic development of *Kordyana brasiliensis* and visible symptoms on each of the test plant taxon inoculated with the fungus in tests conducted in Australia, based on categories described in Figure 6. Susceptibility ratings were evaluated for each test plant taxon according to the categories presented in Table 3.

SPECIES [A]	MICROSCOPIC OBSERVATIONS																	VISIBLE SYMPTOMS				RATING [B]
	GERMINATION			SURFACE HYPHAE	PENETRATION			COLONISATION						REPRODUCTION								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Aneilema acuminatum	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	R
Aneilema biflorum	–	–	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	+	–	–	–	HR
Callisia repens	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Cartonema philydroides	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Commelina ciliate	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Commelina cyanea	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Commelina diffusa	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Commelina aff. *diffusa* (NT)	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	R
Commelina ensifolia	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Commelina lanceolata	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Cyanotis axillaris	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Dichorisandra thysiflora [C]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Floscopa scandens	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Gibasis geniculata	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	HR
Murdannia gigantea	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Murdannia graminea	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Pollia crispate	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	R
Pollia macrophylla	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	R
Tradescantia fluminensis	–	–	+	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	HS

SPECIES [A]	MICROSCOPIC OBSERVATIONS																	VISIBLE SYMPTOMS				RATING [B]
	GERMINATION			SURFACE HYPHAE		PENETRATION			COLONISATION						REPRODUCTION							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Tradescantia sp. Giant leaf (Browns Reserve accession)	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	R
Tradescantia pallida	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia zebrina	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia spathacea	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia ‘Cindy’	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia ‘Isis’	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia ‘J C Wegellin’	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia ‘Nutshell Rosy’	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia ‘Snowflake’	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I
Tradescantia ‘Sweet Kate’	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	I

[A] All taxa were tested in two separate trials (five plant replicates per taxon per trial unless indicated otherwise).
[B] R = Resistant; HR = Highly resistant; I = Immune (see Table 3 for more detail).
[C] Three and four replicates were used in each of the trials for this taxon, respectively.

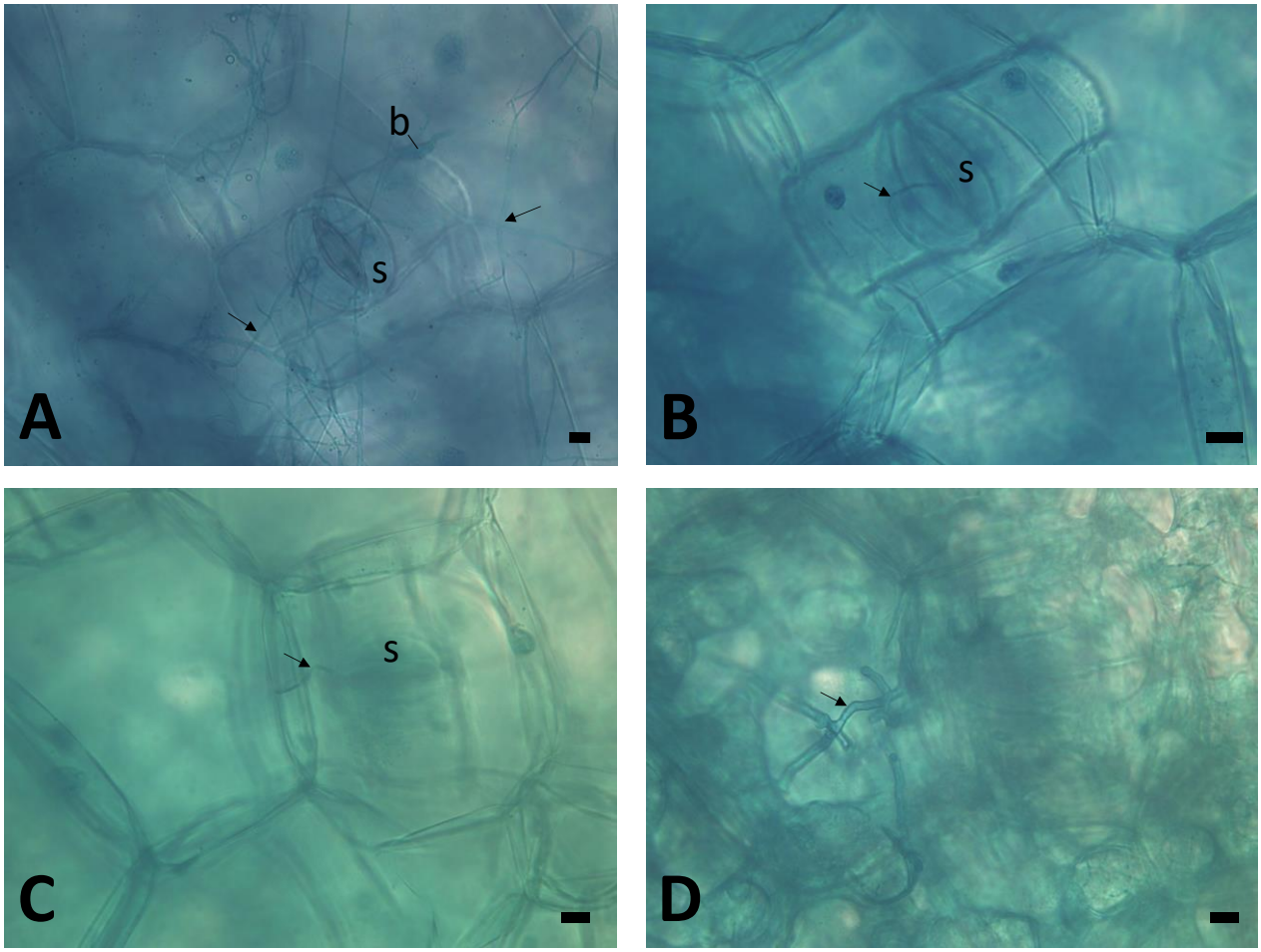

Figure 7 Development of *Kordyana brasiliensis* on leaves of wandering trad (*Tradescantia fluminensis*) (bars = 10 µm) at 5 days after the beginning of the inoculation period. A. Germinated basidiospore (b) and several hyphae (arrows) on the leaf surface, including one hypha growing in the opening of a stomate (s). B. Penetration hypha (arrow) emerging in the cavity below a stomate (s). C & D. Micrographs taken at different depths of field. Penetration hypha (arrow) emerging in the cavity below a stomate (s) (C) and intercellular hyphae (arrow) present deeper in the leaf tissue (D).

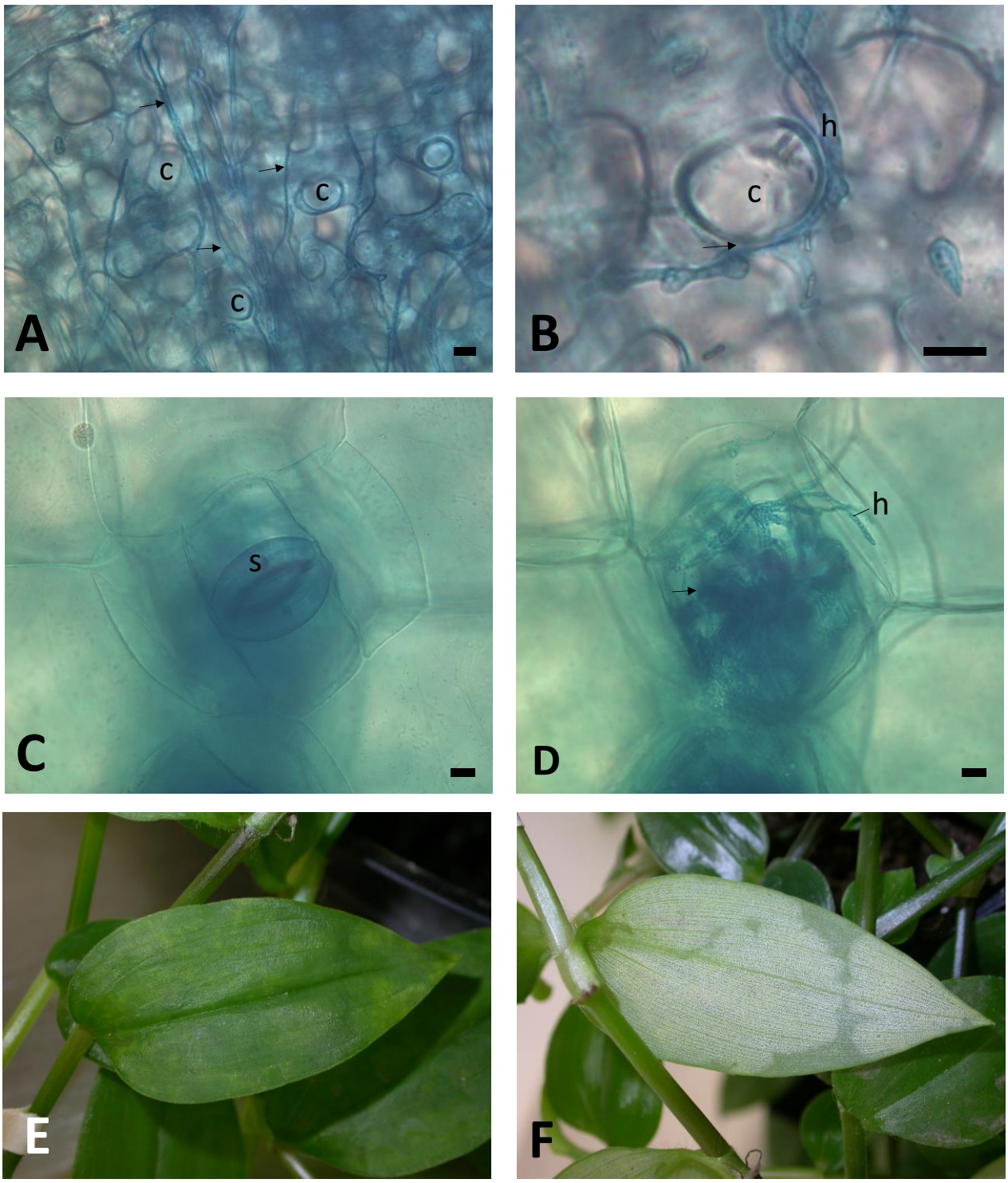

Figure 8 Development of *Kordyana brasiliensis* on leaves of wandering trad (*Tradescantia fluminensis*) (bars = 10 µm) at 28 days after the beginning of the inoculation period. A. Extensive network of hyphae (arrows) growing between plant cells (c) within the leaf tissue. B. Interaction site (arrow) between an intercellular hyphae (h) and a plant cell (c). C & D. Micrographs of the same sample taken at different depths of field. C. Leaf surface with stomate (s); D. sorus with a basidial layer (arrow) that developed from intercellular hyphae (h) in the chamber below the stomate. E. Visible symptoms on the upper surface of a leaf, F. Visible symptoms on the under surface of a leaf, with abundant basidiospores (woolly white appearance) produced from basidia that protrude through stomata.

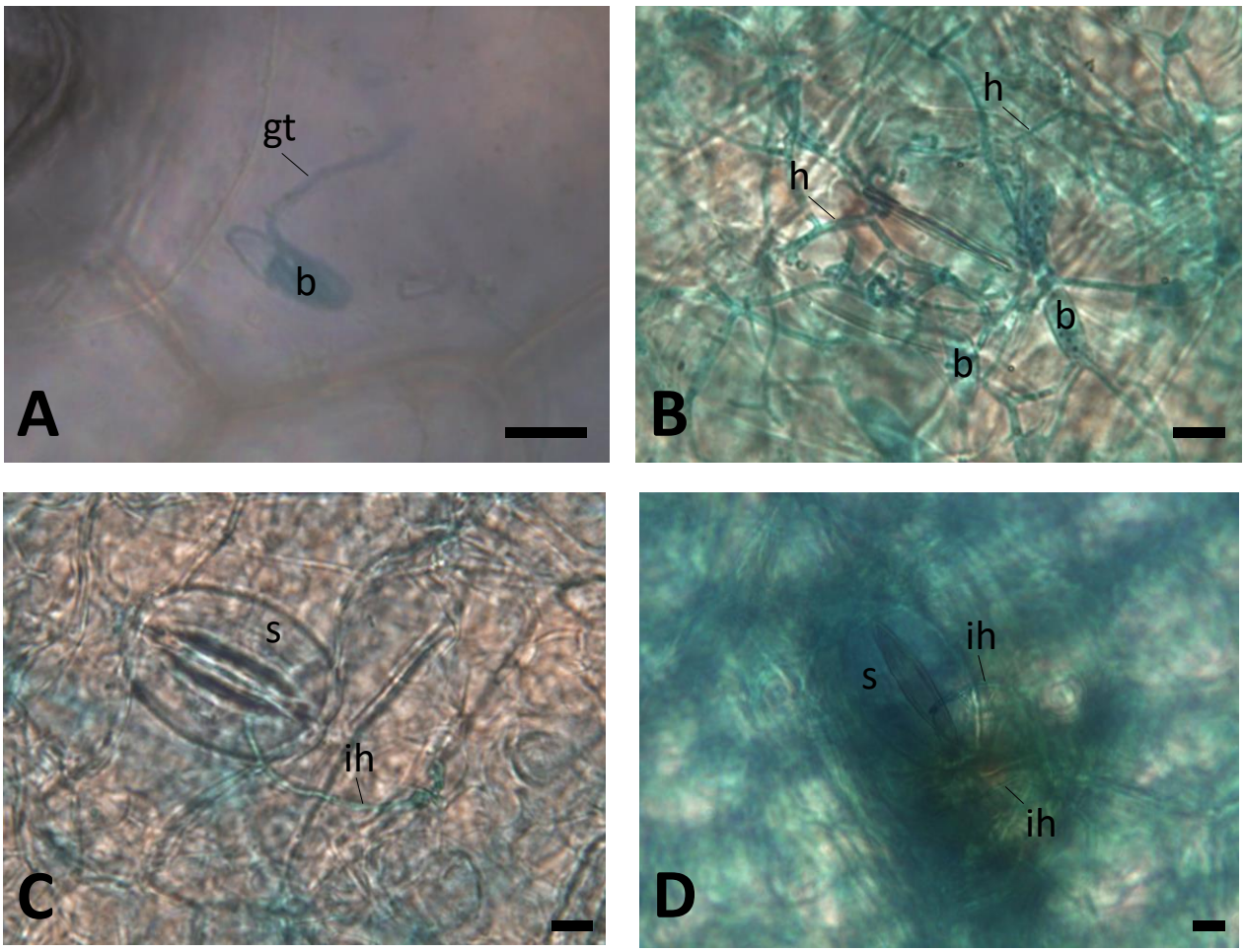

Figure 9 Development of *Kordyana brasiliensis* on leaves of different plant species. A. Germinated basidiospore (b) with germ-tube (gt) on the leaf surface of *Pollia crispata* at 28 days after the beginning of the inoculation (DAI). B. Hyphae (h) growing on the leaf surface of *Commelina lanceolata* at 5 DAI (b= germinated basidiospore). C. Limited intercellular hyphae (ih) development following penetration of a stomate (s) on a *Gibasis geniculata* leaf at 5 DAI. D. Limited intercellular hyphae (ih) development following penetration of a stomate (s) on a *Tradescantia* sp. Giant leaf (Browns Reserve accession) leaf at 28 DAI. Note host cell necrosis associated with the infection site.

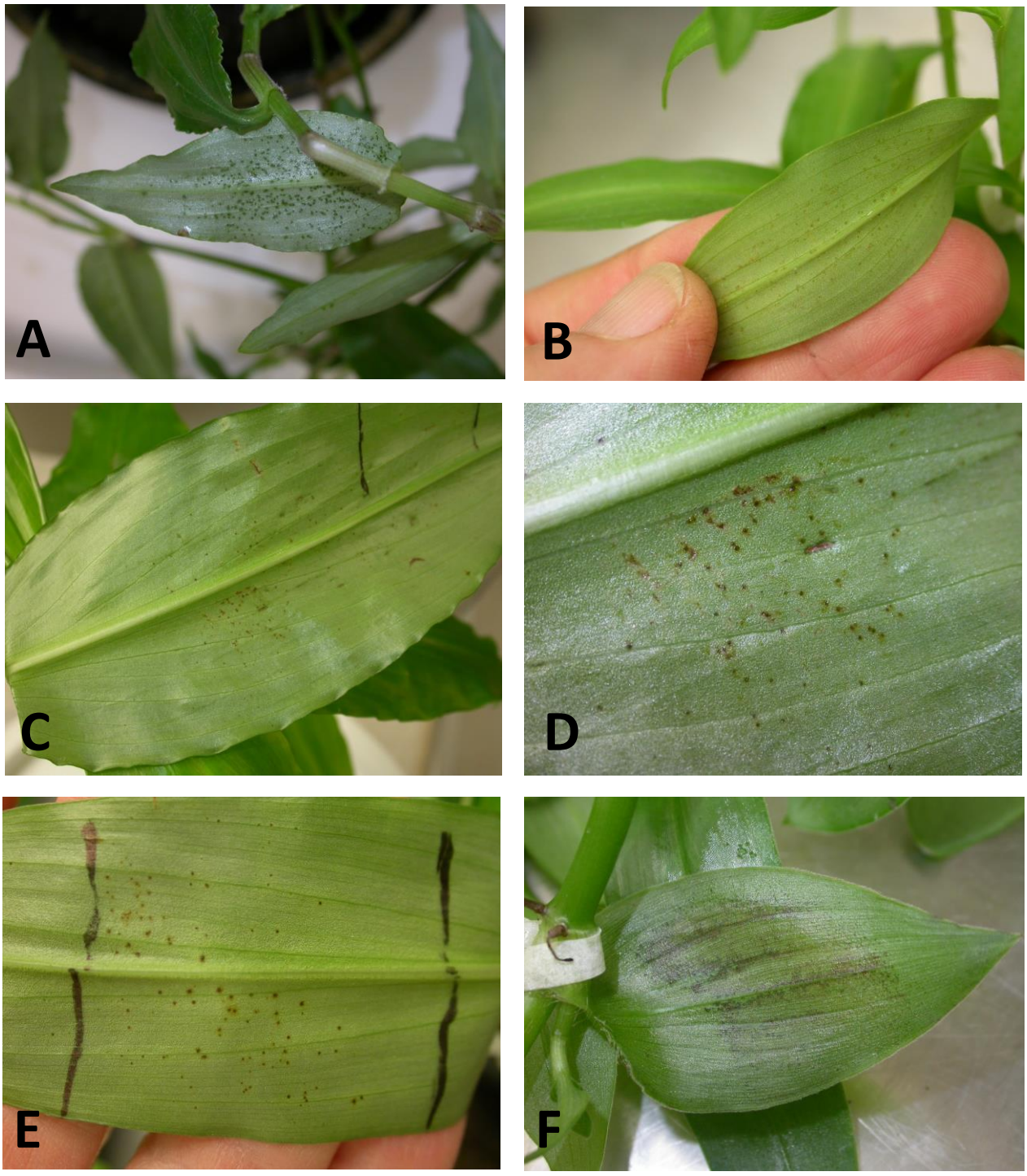

Figure 10 Visible symptoms observed on leaves of different species at 28 days after the beginning of the inoculation with *Kordyana brasiliensis:* A. *Aneilema acuminatum* , B. *Commelina* aff. *diffusa* (NT accession), C & D. *Pollia macrophylla* , E. *Pollia crispata*, F. *Tradescantia* sp. Giant leaf (Browns Reserve accession).

2.10.3 DISCUSSION

Host-specificity testing of a candidate biological control agent can never give an absolute answer as it is impossible to prove a negative hypothesis, i.e. that a plant species will never be attacked. The determination of the likely host-range of an introduced plant pathogen following its release in a new environment and potential exposure to thousands of plant species with which it has never before come in contact, is not an easy process. Some risks are always going to be associated with such movements of living organisms and the fundamental principle of a testing program is to establish that level of risk. If that level is shown to be acceptably low, an introduction can proceed with the confidence that the probability of unexpected negative effects is negligible. The design of test lists for candidate weed biological control agents follows a widely accepted scientific approach, the centrifugal phylogenetic method, which places greater representation on the more closely related species to the target weed (Wapshere 1984, Briese 2003, Sheppard et al. 2005). Host-specificity testing is performed in order to assess risks, and the centrifugal phylogenetic method offers the most rational and logical approach to decide whether one plant species or another should be tested.

The vast majority of organisms attacking plants show a degree of specialisation to their hosts. Biotrophic pathogens, such as *K. brasiliensis*, have elaborate associations with a restricted range of plant species. They must possess many pathogenicity factors, from recognition of the host surface at the time of germination to the production of specific molecules that suppress defence responses in the plant, to establish a compatible interaction with a host plant. All plants appear to have a series of constitutive and inducible features that can protect them against invasion by fungal pathogens. This is generally referred to as 'basic resistance', which is non-parasite-specific. Several plant responses, most elicited by the fungus attempting to invade, have been implicated in basic resistance e.g. constitutive toxic chemicals, synthesis of phytoalexins, chemical modifications of cell walls, hypersensitive reactions. On the other hand, pathogens that possess sufficient pathogenicity factors to suppress or negate the defence mechanisms of a plant will be successful at establishing a compatible interaction with the plant i.e. infect the plant. The matching attributes between the pathogen and the plant result in what is called 'basic compatibility'. The more closely related two plant species are, the greater the probability that they will have biochemical and genetic properties in common. This is the reason why considerable emphasis is placed on phylogenetic relationships between plants in determining a list for testing.

Results from host-specificity tests performed with *K. brasiliensis* in Brazil and Australia indicate that it is a highly host specific fungus. It can only successfully infect wandering trad accessions and develop normal lesions that produce copious amounts of basidiospores. Only five non-target plant taxa developed flecks that were water-soaked in appearance or necrotic following inoculation with the fungus. These flecks never developed further into lesions and thus the fungus could not complete its life cycle on these taxa. These flecks are typical signs of hypersensitive reactions, which are caused by the rapid death of plant cells in the local region surrounding the area where a pathogen has tried to infect. This plant defence mechanism restricts/stops further development by the pathogen.

All other non-target plant taxa tested did not develop any visible symptoms. The fungus did not succeed at infecting any of these taxa under the conditions used during testing, which were optimal for disease development on the susceptible host wandering trad. Such artificial conditions during host-specificity tests have been reported to predispose plants to infection by plant pathogens (e.g. Bruckart et al. 1985).

Microscopic examinations of the infection process of *K. brasiliensis* on its host wandering trad confirmed that the fungus penetrates leaf tissue via stomata following basidiospore germination on the leaf surface and development of surface hyphae. Once within the leaf, it produces intercellular hyphae that attached to host cells to form complex interaction apparatus, as seen in other *Kordyana* species (Bauer et al. 1997, Bergerow et al. 2002). It is through these interaction apparatus that the fungus extracts nutrients from the host for its own growth and reproduction. While similar levels of basidiospore germination and surface hyphae development by *K. brasiliensis* were observed on the leaves of the 28 non-target plant taxa tested in Australia, penetration of stomata and development of some intercellular hyphae were only observed in 6 of the taxa. The limited growth of these intercellular hyphae within leaf tissue and/or development of

water-soaked or necrotic flecks in these taxa are typical reactions following activation of non-specific, basic resistance mechanisms of non-host plants (Gill et al. 2015).

2.11 Conclusion

The high specificity of *K. brasiliensis* demonstrated in the studies conducted in Brazil and Australia indicates that this fungus would not pose a threat to non-target plant species should it be released into Australia.

Kordyana brasiliensis is expected to be a damaging pathogen of wandering trad in Australia. It is highly pathogenic on its host, is capable of infecting all leaves irrespective of their age and can produce large quantities of inoculum since it has a high number of generations during a single growing season. Under optimum conditions, *K. brasiliensis* can complete its life cycle on wandering trad within 2–3 weeks. The dew produced in shady habitats where wandering trad thrives, especially at nights during autumn and winter, should be conducive to the development of severe epidemics by the fungus. *Kordyana brasiliensis* should work in tandem with the three biological control beetles that are being used in the New Zealand biological control program, if they are eventually released in Australia.

In summary, we conclude that the level of risk associated with releasing *K. brasiliensis* is acceptable and that it will be a potentially effective biological control agent against wandering trad across its invaded range. We therefore seek permission for its release in Australia.

References

Any of these references can be provided in electronic form if requested. Contact Louise Morin (louise.morin@csiro.au; Ph: (02) 6246 4355)

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Appendix A Wandering trad: An approved target for biological control

Responses from representatives of each state and territory on the Invasive Plants and Animals Committee to the proposed nomination of wandering trad as a target for biological control. On the basis of these responses, wandering trad was approved as a target for biological control. Contact IPAC secretariat (ipac@agriculture.gov.au) for more details.

Invasive Plants and Animals Committee

Sub-committee of the National Biosecurity Committee

Summary of Responses

IPAC OOS 2015 - 11

TITLE: Nomination of *Tradescantia fluminensis* as a biocontrol target

LEAD: Andrew Woolnough, DEDJTR, Victoria

DATE: 4 November 2015

RESPONSE DUE BY: 18 November 2015

FOR DECISION

Recommendations:
That the Invasive Plants and Animals Committee:

1. **Agrees** that *Tradescantia fluminensis* Vell. be approved as a target for biological control.

Jurisdiction	1
Australian Capital Territory	Agreed – with comment
New South Wales	Agreed – with comment
Northern Territory	Agreed
Victoria	Agreed – with comment
Queensland	Agreed
Western Australia	Agreed – with comment
South Australia	Agreed – with comment
Tasmania	Agreed – with comment
Australian Government	
Department of Agriculture	Agreed
Department of the Environment	Agreed

Jurisdictional Comments

Australian Capital Territory
ACT supports the nomination of *Tradescantia fluminensis* as a target for biological control because:

- it's a problematic invasive weed in a number of jurisdictions including Victoria (the proponent);

- herbicide control is often not appropriate near waterways and manual control is only practical for small areas;
- nomination has in-principle support from Nursery and Garden Industry Australia (there is only minor trade and use in gardens – many gardeners view it as a difficult to control weed);
- there is a biological control program underway in NZ (lots of the work has already been done to identify control agents); and
- there are no native or introduced species in the Commelinaceae in the ACT (http://www.anbg.gov.au/cpbr/ACT-census-2012/index.html#statistics) except probably as house or garden plants.

New South Wales
NSW support this application. Tradescantia is a much maligned common environmental weed particularly around Sydney. The only complication is the other ornamental (purple) variety of the Genus which was popular in the trade in the 70/80s. The prospects for control are very good, with three insect biological control agents established in the field in New Zealand.
I'm sure the industry can cope with its insignificant loss to trade we also support the application.

South Australia
Tradescantia fluminensis is a suitable target for biological control as it is a weed that occurs in dense populations in the eastern States. It is more localised in SA, where biological control may be less applicable. The thorough discussion of phylogenetic relationships and list of Commelinaceae and Cartonemataceae species is adequate. A full test list for a proposed agent would need to include some representatives of more distantly related families as well.
It is not correct to claim (dot point 3) that *Tradescantia fluminensis* is 'not cultivated'. Firstly, this statement is explicitly contradicted by Table 1, p. 10, where cultivars such as Albovittata are mentioned. Secondly, even if a species is not in the commercial trade it may still be grown non-commercially by gardeners – and *T. fluminensis* certainly is. However, this is not sufficient reason to reject it as a target for biological control.

Tasmania
Tasmania requests that it be kept informed in relation to the trialling of any biological agents.

Victoria
Tradescantia appears to be a very good candidate for biological control.

Western Australia
Tradescantia fluminensis is a widespread garden plant in WA, commonly found in older gardens. Its environmental impacts in WA are relatively minor, with it only being found in a few altered wetlands in the Perth area from dumped garden refuse. It is still in cultivation in WA (i.e. private but not in commercial trade).

The New Zealand biocontrol agents may work well there and could be expected to be effective in the wetter, cooler south eastern states where *T. fluminensis* is a major environmental pest and an effective agent could be beneficial.

Appendix B Members of the family Commelinaceae in Australia

Members of the family Commelinaceae native to or naturalised in Australia.

Tribe [A]	Species [B]	Distribution and habitat	Source [C]
Cartonemateae [D]	*Cartonema antrorsum*	NT, QLD	Unpublished, Flora of Aust.
	Cartonema baileyi	QLD	APNI
	Cartonema brachyantherum	QLD	APNI
	Cartonema bulbosum	NT	Unpublished, Flora of Aust.
	Cartonema caespitosum	NT	Unpublished, Flora of Aust.
	Cartonema latifolium	NT	Unpublished, Flora of Aust.
	Cartonema parviflorum [E]	WA, NT, QLD	APNI
	Cartonema pedicellatum	NT	Unpublished, Flora of Aust.
	Cartonema philydroides	WA	APNI
	Cartonema purpureum	WA	Unpublished, Flora of Aust.
	Cartonema spicatum [F]	WA, NT, QLD	APNI
	Cartonema trigonospermum	NT	APNI
Commelineae	*Aneilema acuminatum*	NSW, QLD; rainforest and tall sclerophyll forest	PlantNET
	Aneilema biflorum	NSW, QLD; damp and shaded paces, often near streams	PlantNET
	Aneilema papuanum	QLD	Unpublished, Flora of Aust.
	Aneilema sclerocarpum	QLD	Unpublished, Flora of Aust.
	Aneilema siliculosum	NT, QLD	APNI
	**Commelina africana*	NSW	PlantNET
	Commelina agrostophylla	NT, QLD	APNI
	**Commelina benghalensis*	NSW, QLD, NT, WA, SA	PlantNET

Tribe [A]	Species [B]	Distribution and habitat	Source [C]
	Commelina ciliata	WA, NT, QLD	APNI
	Commelina clarksoniana	QLD	Unpublished, Flora of Aust.
	Commelina cyanea	NSW, QLD; moist forest or woodland	PlantNET
	Commelina diffusa	NT, QLD; reported in Victoria but no voucher specimen	APNI
	Commelina ensifolia [G]	QLD, WA, NT; moist forest or woodland	PlantNET
	Commelina lanceolata	QLD	APNI
	Commelina reticulata	WA	APNI
	Commelina tricarinata	NT, QLD	APNI
	Commelina tuberculata	QLD	Unpublished, Flora of Aust.
	Floscopa scandens	QLD	APNI
	Murdania brevifolia	QLD	Unpublished, Flora of Aust.
	Murdannia cryptantha	NT, QLD; new species described in 1993	APNI
	Murdannia gigantea	NT, QLD	APNI
	**Murdannia keisak*	NSW	APNI, PlantNET
	**Murdannia nudiflora*	WA, NT, QLD	APNI
	**Murdannia vaginata*	NT, QLD	APNI
	Murdannia graminea	NSW, QLD, WA, NT; sclerophyll forest	PlantNET
	Murdannia polygonoides	NT	Unpublished, Flora of Aust.
	Pollia crispata	NSW, QLD; rainforest, or rainforest margins, in wet places or along creeks	PlantNET
	Pollia macrophylla	QLD; rainforest	APNI
	Tapheocarpa calandrinioides	QLD; new genus described in 1994	APNI
Tradescantieae	**Callisia fragrans*	QLD, NSW	APNI
	**Callisia repens*	QLD	APNI
	Cyanotis axillaris	WA, NT, QLD	APNI
	**Dichorisandra thyrsiflora*	Far north NSW, SE QLD	PlantNET
	** Gibasis pelludica*	NSW	PlantNET
	**Tradescantia cerinthoides*	NSW, SA	PlantNET

Tribe [A]	Species [B]	Distribution and habitat	Source [C]
	**Tradescantia fluminensis*	WA, SA, QLD, NSW, LHI, VIC, TAS	APNI
	**Tradescantia pallida*	NSW, QLD	PlantNET
	**Tradescantia zebrina*	QLD, NSW; rainforest coastal sites	PlantNET
	**Tradescantia spathacea*	NSW, QLD.	PlantNET

[A] Tribe based on combined rbcL and morphology datasets of Evans et al. (2003), where one (to three) species were used to represent each genus.

[B] Introduced species are denoted by '*'.

[C] 'Unpublished, Flora of Aust.' indicates that these species are only listed in the draft chapter on Commelinaceae for the Flora of Australia, provided by the expert taxonomist from The University of Adelaide. The other sources are where information on distribution and habitat was obtained: APNI = Australian Plant Name Index (http://www.anbg.gov.au/apni/). PlantNET (http://plantnet.rbgsyd.nsw.gov.au/).

[D] Sister to the other tribes of the family in Australasia.

[E] *Cartonema tenue* is a synonym of *C. parviflorum*.

[F] *Cartonema brachyantherum* is a synonym of *C. spicatum* subsp. *humile* (Taxonomist, The University of Adelaide, pers. comm.).

[G] *Commelina undulata* is a synonym of *C. ensifolia* (Taxonomist, The University of Adelaide, pers. comm.).

Appendix C Host-specificity tests with *Kordyana brasiliensis* in Brazil

METHODOLOGY

Non-target plants used in host-specificity tests were purchased from nurseries in Brazil, wherever possible (Universidade Federal de Viçosa staff, pers. comm.). Propagating material of an accession of wandering trad (*T. fluminensis*) was collected from the field in New Zealand and imported into Brazil for the tests.

Two different methods were used for host-specificity tests (Barreto et al. 2010, Barreto and Macedo 2011, Fowler et al. 2013). The first trial used the 'shadehouse method'. This involved placing three potted plants (replicates) of non-target species, including the New Zealand biotype of wandering trad, in rows within a shadehouse at the University of Viçosa. Each row was separated by 50-cm from a row of wandering trad plants (ex. Brazil) with severely disease symptoms of *Kordyana brasiliensis*, as a result of natural infections. Plants were examined weekly for disease symptoms for a month, and then at monthly intervals for the remaining 6 months.

The second and third trial used a direct inoculation method ('spore-drop method'). Wandering trad leaves with lesions of *K. brasiliensis* were collected from the field and their adaxial surface attached to a sheet of glass coated with vaseline, leaving the sporulating abaxial surface exposed. The glass sheet was then placed 60 cm above the test and control (NZ wandering trad) plants (two replicates per species per trial) in a dew chamber for 48 hours at 22°C. After the inoculation period, plants were transferred to a glasshouse at 25°C. Plants were examined weekly for disease symptoms for a total of 65 days.

RESULTS

Results from all three trials using either the shadehouse or spore-drop methods demonstrated that *K. brasiliensis* is highly specific (see Table below). Wandering trad plants (ex. NZ) developed typical symptoms of the disease within one month after they were placed in the shadehouse in the vicinity of plants naturally infected with *K. brasiliensis*. None of the other test plants became infected during the trial.

In the two trials that used the spore-drop method, disease symptoms of *K. brasiliensis* were observed on wandering trad plants (ex. NZ) by 18 days after inoculation. No disease symptoms developed on any of the other plant species for the duration of the trials.

Results from host-specificity tests performed with *Kordyana brasiliensis* in Brazil (+ = infected, – = not infected). Blanks indicate that the plant species was not included in the trial. Results reported in Fowler et al. (2013) and summarised in the application for release of the fungus in New Zealand submitted to the Environmental Protection Authority. [8]

Species	**Trial 1 (shadehouse method)**			**Trial 2 (spore-drop method)**		**Trial 3 (spore-drop method)**	
	Replicate no.			**Replicate no.**		**Replicate no.**	
	1	**2**	**3**	**1**	**2**	**1**	**2**
Tradescantia fluminensis (ex NZ)	+	+	+	+	+	+	+
Tradescantia pallida	–	–	–	–	–	–	–
Tradescantia spathacea	–	–	–	–	–	–	–
Tradescantia zebrina	–	–	–	–	–	–	–
Tradescantia zononia	–	–	–	–	–	–	–
Callisia repens	–	–	–	–	–	–	–
Callisia warszewicziana	–	–	–	–	–	–	–
Gibasis schiediana	–	–	–	–	–	–	–
Tripogandra diuretica	–	–	–	–	–	–	–
Dichorisandra thyrsiflora	–	–	–	–	–	–	–
Siderasis fuscata	–	–	–	–	–	–	–
Tinantia sp.	–	–	–	–	–	–	–
Commelina benghalensis	–	–	–	–	–	–	–
Commelina diffusa	–	–	–	–	–	–	–
Commelina erecta	–	–	–	–	–	–	–
Eichhornia crassipes	–	–	–				
Cyperus rotundus	–	–	–				
Juncus sp.	–	–	–				
Strelitzia reginae	–	–	–				
Hedychium coronarium	–	–	–				
Zingiber officinale	–	–	–				

[8] http://www.epa.govt.nz/search-databases/HSNO%20Application%20Register%20Documents/APP201362_APP201362%20-%20EPA%20Staff%20advise%20report.pdf

Appendix D Australian accessions of wandering trad tested with *Kordyana brasiliensis*

State	Accession name	Location details
VIC	Cann River	On the banks of Cann River
VIC	Wodonga	Felltimber Creek, Wodonga
VIC	Mullum Creek	Mullum Creek, Ringwood, beside the Leonard St Bridge, Melbourne
VIC	Dandenong	Dandenong Creek, Jells Park, Wheelers Hill
VIC	West Road	West Road, 50m south of Clayton Hill Rd., Langwarrin South
NSW	Kangaroo Valley	186B Gerringong Creek Road, Kangaroo Valley
NSW	Coffs Harbour	Pine Brush Creek at Korora, about 4kms north of Coffs Harbour
NSW	Lord Howe Island	Specific details not provided. General Long/Lat used
NSW	Uralla	Lookout area, Mt Mutton, Uralla (CANB725207.1)
NSW	Dungowan	Over road from Dungowan tip
NSW	Sydney	Pennant Hill
SA	Adelaide	Brown Hill Creek, Suburban Mitcham, Adelaide
QLD	Atherton	Curtain Fig National Park, near Yungaburra, Atherton region
QLD	Gold Coast	Deepak Drive, Rufous Whistler park, Willow Vale

Appendix E Source of each plant taxa tested in Australia and observations made at the end (4 week after inoculation) of stages 1 and 2.

Five replicate plants of each taxon (unless otherwise indicated) was tested in two different trials, with the target weed wandering trad (*Tradescantia fluminensis*) included as positive control. The different taxa were propagated from whole plants or foliage collected from a field population by collaborators or from nurseries. One taxon (*Cartonema philydroides*) was grown from seed purchased from a commercial supplier.

Relationship to target weed	Trial	Non-target taxon	Source	Collector	Stage 1: Symptoms on single leaf at 4 wai	Stage 2: Symptoms on leaves at 4 wai
Same genus	1	*Tradescantia* sp. Giant leaf	Field - Melbourne	Tony Dugdale	Necrotic flecks on the inoculated leaf.	Several necrotic flecks on undersurface of one leaf of one rep (different to the plant using in Stage 1).
	2	*Tradescantia* sp. Giant leaf	ditto		Very few small necrotic flecks on the inoculated leaf.	Several small necrotic flecks on undersurface of many of leaves of four reps. Fifth rep with no visible symptoms.
	1	*Tradescantia pallida*	Field - Northern NSW	John Hosking	none	none
	2	*Tradescantia pallida*	ditto		none	none
	1	*Tradescantia spathacea*	Field - Lord Howe Island	Sue Bower	none	none
	2	*Tradescantia spathacea*	ditto		none	none
	1	*Tradescantia zebrina*	Field - Lord Howe Island	Sue Bower	none	none
	2	*Tradescantia zebrina*	ditto		none	none
	1	Tradescantia 'Cindy'	ornamental trade (Nutshell Nursery)		none	none
	2	Tradescantia 'Cindy'	ditto		none	none
	1	Tradescantia 'Isis'	ornamental trade (Nutshell Nursery)		none	none
	2	Tradescantia 'Isis'	ditto		none	none
	1	Tradescantia 'J C Wegellin'	ornamental trade (Nutshell Nursery)		none	none

	2	Tradescantia 'J C Wegellin'	ditto		none	none
	1	Tradescantia 'Nutshell Rosy'	ornamental trade (Nutshell Nursery)		none	none
	2	Tradescantia 'Nutshell Rosy'	ditto		none	none
	1	*Tradescantia* 'Snowflake'	ornamental trade (Nutshell Nursery)		none	none
	2	*Tradescantia* 'Snowflake'	ditto		none	none
	1	Tradescantia 'Sweet Kate'	ornamental trade (Melbourne nursery)		none	none
	2	Tradescantia 'Sweet Kate'	ditto		none	none
Same sub-tribe	1	*Callisia repens*	Field - Gold Coast, QLD	Liz Snow	none	none
	2	*Callisia repens*	ditto		none	none
	1	*Gibasis geniculata*	ornamental trade (Bunnings)		none	none
	2	*Gibasis geniculata*	ditto		none	none
Same tribe	1	*Cyanotis axillaris*	Field - North Queensland	Rigel Jensen	none	none
	2	*Cyanotis axillaris*	ditto		none	none
	1	*Dichorisandra thysiflora* (3 reps)	ornamental trade (Quicksales.com.au)		none	One rep died in the last week of the trial. No symptoms on leaves.
	2	*Dichorisandra thysiflora* (4 reps)	ditto		none	none
Same family	1	*Aneilema acuminatum* (NSW)	Field - Kangaroo Valley, NSW	Les Mitchell	At 9 dai water soaked flecks on the inoculated leaf; same symptoms at 4wai	Small necrotic flecks on undersurface of several leaves across all reps.
	2	*Aneilema acuminatum* (QLD)	Field - North Queensland	Andrew Ford		Water soaked flecks on undersurface of very few leaves in 3 reps and light chlorosis on the uppersurface.
	1	*Aneilema biflorum*	Field - Kangaroo Valley, NSW	Tess Heighes	none	none
	2	*Aneilema biflorum*	ditto		none	none
	1	*Cartonema philydroides*	ornamental trade (Nindethana Seed)		none	none
	2	*Cartonema philydroides*	ditto		none	none
	1	*Commelina ciliata*	Field - North Queensland	Andrew Ford	none	none
	2	*Commelina ciliata*	ditto		none	none

	1	*Commelina cyanea*	Field - Northern NSW	John Hosking	none	none
	2	*Commelina cyanea*	ditto		none	none
	1	*Commelina diffusa*	Field - North Queensland	Andrew Ford	none	none
	2	*Commelina diffusa*	ditto		none	none
	1	*Commelina* aff. *diffusa* (NT)	Field - Darwin, Northern Territory	Raelene Kwong & Ian Cowie	none	none
	2	*Commelina* aff. *diffusa* (NT)	ditto		Small water soaked flecks on the inoculated leaf.	A few watersoaked flecks on undersurface of a few leaves in one rep. No symptoms on other reps.
	1	*Commelina ensifolia*	Field - North Queensland	Andrew Ford	none	none
	2	*Commelina ensifolia*	ditto		none	none
	1	*Commelina lanceolata*	Field - North Queensland	Andrew Ford	none	none
	2	*Commelina lanceolata*	ditto		none	none
	1	*Floscopa scandens*	Field - North Queensland	Barbara Maslen	none	none
	2	*Floscopa scandens*	ditto		none	none
	1	*Murdannia gigantea*	Field - North Queensland	Andrew Ford	none	none
	2	*Murdannia gigantea*	ditto		none	none
	1	*Murdannia graminea* (QLD)	Field - North Queensland	Andrew Ford	none	none
	2	*Murdannia graminea* (NSW)	Field - Northern NSW	John Hosking	none	none
	1	*Pollia crispata*	Field - NSW South Coast	Les Mitchell	Small necrotic flecks on the inoculated leaf.	Very few small necrotic flecks on undersurface of a few leaves on all reps.
	2	*Pollia crispata*	ditto		Several small necrotic flecks on the inoculated leaf.	A few small necrotic flecks on undersurface of some leaves of two reps. Necrotic flecks only on one leaf in another rep. Only very few necrotic flecks on several leaves of the remaining two reps.
	1	*Pollia macrophylla*	Field - North Queensland	Andrew Ford		Small necrotic flecks on undersurface of some leaves of one rep.
	2	*Pollia macrophylla*	ditto		Small necrotic flecks on the inoculated leaf.	Very few small necrotic flecks on undersurface of leaves of 3 reps. No symptoms on other reps.

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FOR FURTHER INFORMATION

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