Confirmation of *Itersonilia perplexans* infecting pyrethrum (*Tanacetum cinerariifolium*) in Australia

Stacey Pilkington[1], Jason Scott[1], Tamieka Pearce[1], Yu Pei Tan [2], Frank Hay[1*]

[1]Tasmanian Institute of Agriculture, P.O. Box 3523, Burnie, Tasmania 7320, Australia

[2]Queensland Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, Queensland 4102, Australia

*current address: Cornell University, School of Integrative Plant Science, Section of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY 14456, USA

Author for correspondence: Jason Scott, E-mail: Jason.Scott@utas.edu.au

Acknowledgements

This research was funded by Hort Innovation with co-investment from Botanical Resources Australia – Agricultural Services Pty. Ltd (BRA) and funds from the Australian Government through project PY12001. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture

Pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.) is grown to extract pyrethrins which are active ingredients for insecticides (Greenhill 2007). The Australian pyrethrum industry supplies over 50% of the world market. Surveys of Tasmanian crops in spring 2013, detected the presence of a fungus putatively identified as *Itersonilia perplexans* Derx. on foliage in 54 of 86 surveyed fields (Hay et al. 2015). This fungus was associated with necrotic leaf tips often spreading to encompass whole leaves. However, pathogenicity to pyrethrum was not confirmed.

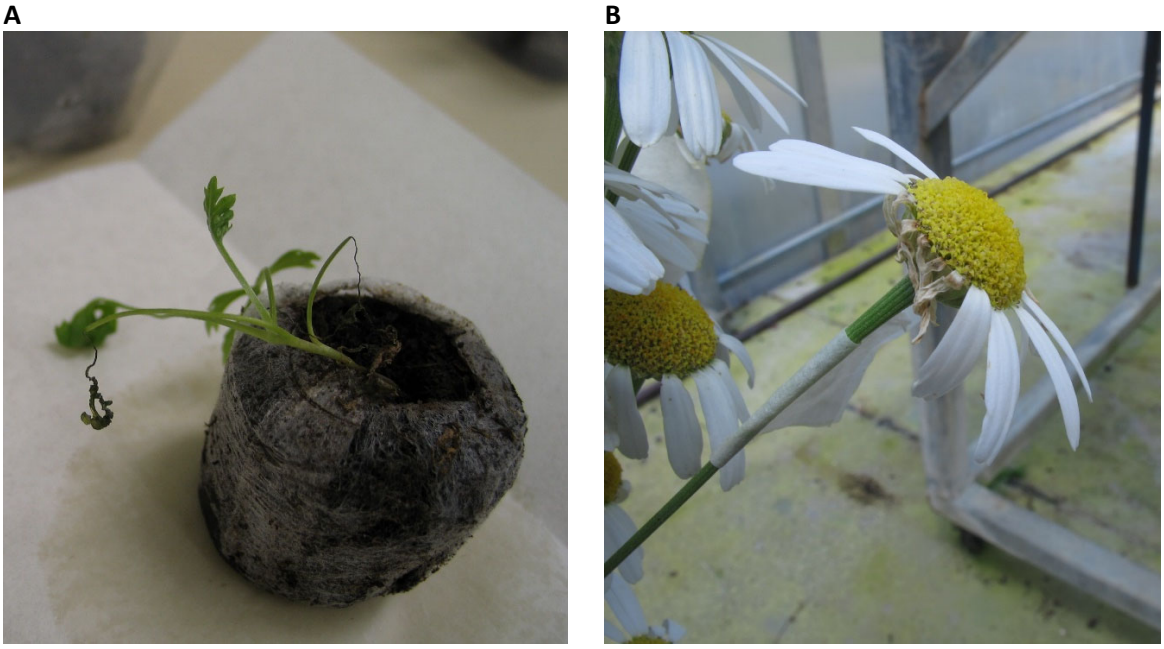
To isolate, tissue was excised from foliar lesions, surface sterilised using 0.4% NaClO, placed onto 2% water agar and incubated at 20°C for 5 days. Colonies were pure-cultured by hyphal-tip transfer onto potato-dextrose agar. Eleven isolates were cultured onto yeast mold agar (YMA) for 14 days at 15°C in the dark (Horita and Yasuoka 2002). Colonies were slow growing (1.9 to 2.3 mm/day) white to buff on both surfaces, with a darker center visible on lower surfaces. Mycelia were straight and hyaline with clamp connections at the septa. Squares transferred from the edge of YMA colonies onto microscope slides produced ballistoconidia that were aseptate, granular and lunate, kidney or lemon-shaped after 24 h. Ballistoconidia lengths and widths (n = 50/isolate) ranged from 14.6 to 20.4 µm and 10.0 to 13.6 µm. Chlamydospores were not observed. These observations were

consistent with descriptions of *I. perplexans* (Koike and Tjosvold 2001; Liu et al. 2015). All 11 isolates were sequenced across the internal transcribed spacer (ITS) region of rDNA (ITS; primers V9G/ITS4; de Hoog and van den Ende 1998; White et al. 1990), and large (LSU; primers LROR/LR7; Rehner and Samuels 1995), and small (SSU; NS1/NS4; White et al. 1990) subunits of rDNA (Genbank accession nos. KU563626 to KU563658). The ITS (673 bp), SSU (1,047 bp) and LSU (1,318 bp) differed by 3, 1 and 0 bp, respectively, across isolates. Maximum parsimony and maximum likelihood analyses of a concatenated 3 loci alignment with Cystofilobasidiales representatives (Liu et al. 2015) placed all isolates and the *I. perplexans* ex-neotype strain CBS 363.85 within a single monophyletic clade with 100% bootstrap support. Two representative isolates are stored at the Plant Pathology Herbarium (accession nos. BRIP 57986 and 57987).

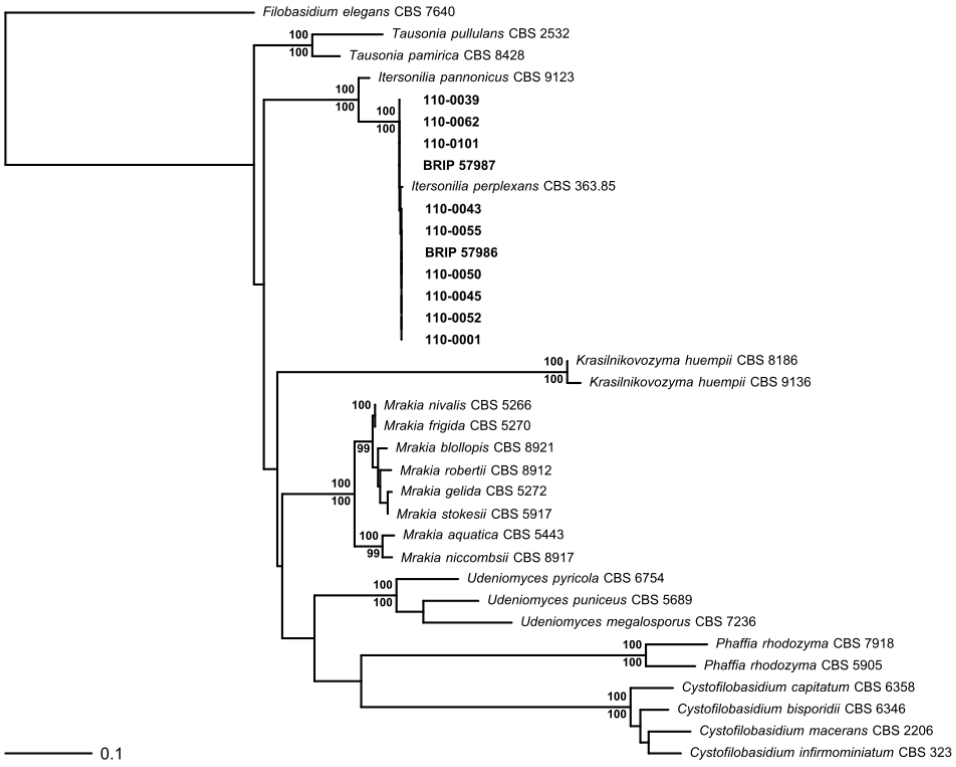
Leaves of 46-day-old pyrethrum plants (*n* = 45), generated from surface sterilised seed, were inoculated with a 1.5×10^5 ballistoconidia/ml suspension (equal mix of eight isolates) and maintained between 10 and 22°C under a 12-h photoperiod for 14 days. Brown necrotic leaf tips, consistent with reported field symptoms were observed on 71% of plants and *I. perplexans* was recovered from 69% of symptomatic plants. For flower inoculations, pyrethrum plants were removed from fields as vegetative plants in spring and maintained in a greenhouse set at 20:14°C and 14:10 h day:night. Open flowers (10 per plant) were dipped into a 1.2×10^4 ballistoconidia/ml suspension mix of three isolates. Brown withered ray florets were observed on 10/12 plants 18 days post-inoculation, matching those described in petal blight of chrysanthemum (McRitchie et al. 1973). *I. perplexans* was re-isolated from 11/12 inoculated plants and 1 control plant (of 12) which exhibited the same symptoms. In both experiments, *I. perplexans* was identified based on its distinctive morphology. This confirms the pathogenicity of *I. perplexans* to both pyrethrum leaves and flowers.

References

de Hoog, G. S., and van den Ende, A. H. G. G. 1998. Mycoses **41**:183-189.
Greenhill, M. 2007. Chronica Horticulturae **47**:5-8.
Hay, F. S., et al. 2015. Plant Dis. **99**:1227-1335.
Horita, H., and Yasuoka, S. 2002. J. Gen. Plant Pathol. **68**:277-283.
Koike, S. T., and Tjosvold, S. A. 2001. Plant Dis. **85**:802.
Liu, X. Z., et al. 2015. Stud. Mycol. **81**:85-147.
McRitchie, J. J., et al. 1973. Plant Disease Reporter **57**:181-182.
Rehner, S. A., and Samuels, G. J. 1995. Can. J. Bot. **73**:S816-S823.
White, T. J., et al. 1990. Pages 315-322 in: PCR Protocols: A guide to methods and applications. Academic Press, Inc., San Diego, CA, USA.



Supplementary figure 1 Typical symptoms of infection of pyrethrum by *Itersonilia perplexans*. A) necrosis of leaf tips. B) Brown withered ray florets.



Supplementary figure 2 Phylogenetic tree of Cystofilobasidiales representatives (Liu et al. 2015) including putative *Itersonilia perplexans* isolates isolated from Tasmania pyrethrum fields (bold text). Tree constructed from a concatenated alignment of sequences from the large subunit, small subunit and intergenic spacer gene regions. Tree created using maximum likelihood using a general time reversible model with gamma distribution with RAxML. Bootstrap support was generated from 1,000 bootstrap replicates (above branches). Bootstrap values below branches indicate support obtained from 1,000 bootstrap replicates under maximum parsimony analysis. Bootstrap values greater than 90% are shown.