Should we be concerned about rust-resistant biotypes of *Hypericum androsaemum* L. (tutsan) in south-eastern Australia?

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Summary   *Hypericum androsaemum* L. (tutsan) invades pastures and natural ecosystems in high rainfall areas of south-eastern Australia. The rust fungus *Melampsora hypericorum* was detected in Australia in 1991 and drastically reduced the extent of *H. androsaemum* populations in some areas. The impact of the rust is variable and host resistance is present within populations of *H. androsaemum*. Dense populations of *H. androsaemum* reduce native species richness, particularly herbs, grasses and sub-shrubs. The selection of virulent strains of the tutsan rust or other biological control agents is required, particularly for the Blue Mountains region of NSW, where *H. androsaemum* threatens high value biodiversity assets, and is resistant to rust strains that have been highly effective in Victoria.

Keywords   Biocontrol, rust *Melampsora*, resistance.

INTRODUCTION

Hypericum androsaemum L. (Clusiaceae) is a semi-deciduous, soft-wooded sub-shrub native to western and southern Europe and northern Africa. *H. androsaemum* is shade-tolerant and most commonly found on south-facing slopes of forested areas with high rainfall, warm temperatures and moderate to high humidity (Parsons and Cuthbertson 2001). Dense populations of *H. androsaemum* are reported to reduce the grazing value of pasture, and reduce or eliminate native plant species (Parsons 1973). However, there are no quantitative data available for impacts on biodiversity.

Herbicide and mechanical control options are available for *H. androsaemum*, but are mostly unsuitable for use in native vegetation due to non-target damage and the extent of invasion. Biological control is now the only long-term sustainable means of reducing the spread and impact of *H. androsaemum* (Gourlay *et al*. 2014).

In 1991, the rust fungus *Melamspora hypericorum* (DC.) J.Schröt. (tutsan rust), an unintentional introduction, was first detected in Australia at Apollo Bay in Victoria (Bruzzese and Pascoe, 1992). Although variable in its impact, the rust caused a spectacular decline

of *H. androsaemum* in the Otway Ranges of Victoria (McLaren *et al.* 1997). Tutsan rust is a host specific, microcyclic fungus with only one known spore stage, uredinia (Casonato *et al.* 1999). Recorded hosts are a small group of closely related *Hypericum* species: *H. androsaemum*, *H. calycinum* and *H. × inodorum*, although verification is required for species other than *H. androsaemum* (Baker 1955, Casonato 1998). The impact of tutsan rust on *H. androsaemum* is highly variable. No significant relationship could be found between the presence of tutsan rust in the field and phylogenetic grouping based on the ITS sequences of *H. androsaemum* populations (Nel 2012) or random markers (Casonato 1998). By analogy with other rusts, the lack of infection in some populations of *H. androsaemum* is attributed to genetically determined variation in rust virulence and to host susceptibility, possibly due to host polyploidy (Gourlay *et al*. 2014) and multiple introductions into Australia (Casonato 1998, Casonato *et al*. 1999). Similarly, genetic variation in Australian populations of *M. hypericorum* has been detected and may represent more than one introduction of the rust (Casonato 1998, Nel 2012).

Identifying genetic markers for either host resistance (*r* gene) or pathogen virulence (*avr* gene) could explain variation in virulence of different strains of rust on different provenances of *H. androsaemum*, and lead to the selection of effective rust strains for biological control. However, multiple attempts to identify these markers have so far been unsuccessful (Casonato 1998, Nel 2012, Landcare Research 2011, H. Gourlay pers. comm. 2016) and the molecular methods used so far do not target specifically the genes for virulence in the fungus or resistance in the host. An international collaborative program led by Landcare Research NZ is attempting to find tutsan rust strains or other potential agents that have a greater impact on *H. androsaemum* in New Zealand (Landcare Research 2011, H. Gourlay, Landcare Research NZ, pers. comm.). Investment in further biological control in Australia is contingent on understanding the impacts of *H. androsaemum* in natural ecosystems (R. Carter, Central Tablelands Local Land Services, pers. comm. 2014). The impact

of weeds on biodiversity values are not well documented in Australia and reports for *H. androsaemum* are anecdotal and unsubstantiated.

In this paper we investigate the impact of *H. androsaemum* on plant species richness in the Jenolan Caves area within the Jenolan Karst Conservation Reserve, New South Wales, an area heavily invaded by *H. androsaemum*.

MATERIALS AND METHODS
The study site was located on steep south or south-east facing slopes adjacent to the Jenolan River in Jenolan Karst Conservation Reserve, NSW. The vegetation community in the study site was sheltered grey gum forest or sheltered narrow-leaf peppermint forest. In November 2015, circular quadrats (4 m diameter) were haphazardly located in areas invaded by *H. androsaemum*. Sampling points were located in areas with uniform density of *H. androsaemum* and within patches exceeding 50 square metres. Sites with varying densities of *H. androsaemum* were intentionally selected. The positions of sample sites were recorded using GPS (UTM GDA94) and the aspect measured. At each sample site, all vascular plants were recorded and a Domin scale cover-abundance ranking was allocated to each species. The above-ground biomass of *H. androsaemum* was harvested from a 1 m² quadrat located in the centre of each sample area. Harvested material was air-dried at 40–60°C for several weeks in a closed polyhouse, before being weighed.

RESULTS
Hypericum androsaemum reduced native species richness and exotic species richness as cover-abundance of *H. androsaemum* increased. Polynominal functions best described the trend line between species richness and *H. androsaemum* cover, with declines in native species richness becoming evident when cover values of *H. androsaemum* exceeded 25% (cover class value 5 or higher) (Figure 1). Where *H. androsaemum* cover is greater than 90%, native species richness was reduced by around 66%, where mostly shade tolerant herbs e.g. (*Stellaria flaccida* Hook*, Urtica incisa* Poir.*, Geranium* sp.*, Hydrocotyle laxiflora* DC.), grasses (*Microlaena stipoides* (Labill.) R.Br.) and ferns (*Adiantum aethiopicum* L.*, Pellaea falcata* (R.Br.) Fée*, Asplenium flabellifolium* Cav.) persisted at low cover-abundance levels. Increasing cover of *H. androsaemum* also lead to decreased cover of exotic plant species (Figure 2).

A strong polynomial relationship was found between the dry weight of *H. androsaemum* and native species richness ($r^2 = 0.831$). Native species richness declined with increasing biomass of *H. androsaemum*,

but reached a basal asymptote of around 10 species^{-2} (Figure 3). Exotic species richness was weakly correlated with the dry mass of *H. androsaemum* (y = $2E - 05x^2 - 0.0153x + 5.29$, $R^2 = 0.5088$)*,* and also declined with increasing biomass of the latter.

Increasing cover of *H. androsaemum* had variable impact on life-form groups. Grasses, herbs and subshrubs showed weak trends (low r^2 values), with cover declining with increasing cover of *H. androsaemum*.
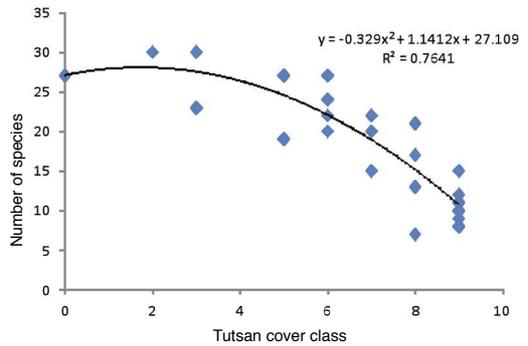

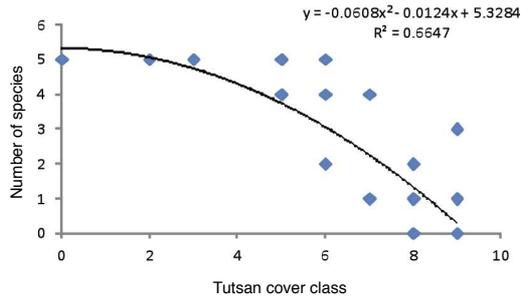**Figure 1.** Native plant richness and cover class of *H. androsaemum*.

Figure 2. Number of exotic species and Domin cover class of *H. androsaemum*.
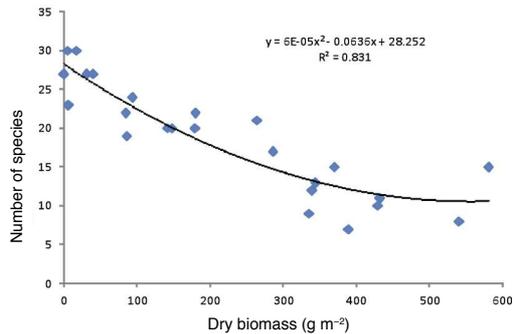

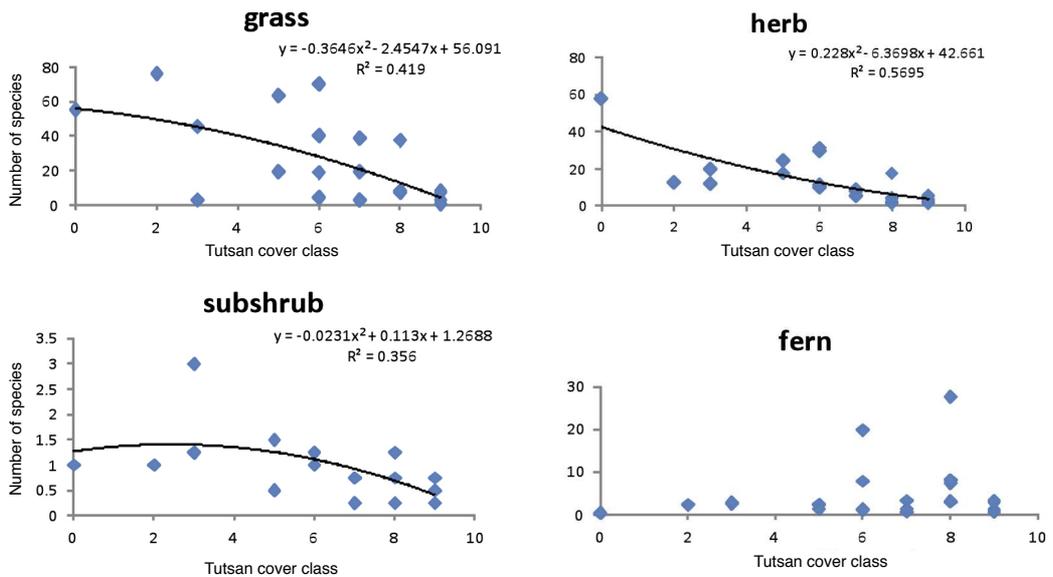**Figure 3.** Plant species richness and dry biomass of *H. androsaemum*.

Figure 4.   Richness of life forms and cover of *H. androsaemum*.

However, no clear trends were evident for shrubs, trees, ferns or lianes (Figure 4).

DISCUSSION

The discovery of tutsan rust in Victoria in 1991 and its subsequent impact on *H. androsaemum* is claimed as the most spectacularly successful example of weed biological control ever witnessed in Victoria (McLaren *et al.* 1997). In a short period of time infestations of *H. androsaemum* at Apollo Bay were drastically reduced in extent and density. Rusts generally have high levels of host specificity, long-distance dispersal capabilities and can severely reduce host fitness when virulent. Therefore, they have considerable potential as classical biological control agents. Weed species with narrow genetic variation make good targets for biological control with rusts (and other agents) as there is a lower probability for the development of host resistance.

The current strains of tutsan rust in Australia provide inadequate control to all populations of *H. androseamum*. In New South Wales, *H. androsaemum* demonstrates resistance to tutsan rust collected from areas in Victoria where control has been effective (Adair 2014). Regrettably, resistant populations of *H. androsaemum* are also emerging in Victoria (Adair 2014).

Our data indicate that native species richness in wet forests of the Blue Mountains is adversely affected by invasion of *H. androsaemum*, and that even low levels of biomass contribute to species decline.

While *H. androsaemum* canopy cover could follow a similar trend it was not evident in our data and appeared to require a threshold cover level of around 50% before native species decline was evident. However, this could reflect lower sensitivity of categorical data (cover class) in detecting trends in the response variable (species richness). The mechanism of species depletion in areas invaded by *H. androsaemum* are not fully understood, but are most likely intense shading and lack of sufficient light to support ground stratum communities of grasses, herbs and sub-shrubs. Reduced availability of water and competition for nutrients may also be involved. While native species may persist at sub-threshold levels, *H. androsaemum* may form a complete canopy cover in susceptible habitats with time, therefore broadening its ecological impact. It is likely that the tree regeneration may be restricted to gaps in the canopy of *H. androsaemum* or areas where *H. androsaemum* is absent or has low cover, but this observation remains speculative until further data is available.

H. androsaemum is widely established along the Jenolan and lower Coxs River catchments in the Blue Mountains and forms dense and extensive cover on south-facing slopes in steep and rugged terrain. Infestations occur within the Greater Blue Mountains World Heritage Area, a biodiversity hotspot (Williams *et al.* 2011), specifically the Kanangra-Boyd Wilderness Area, the Warragamba Special Area, the Blue Mountains and Kanangra-Boyd National Parks, and

the Jenolan Karst Conservation Reserve. *H. androsaemum* has spread from Jenolan Caves to the foreshores of Lake Burragorang (Sydney's main water supply), via the Jenolan and Coxs Rivers.

Recently, a population was discovered in the Hollander's River catchment, which drains to the Kowmung River, a Wild and Scenic river. Currently, *H. androsaemum* threatens restricted plant communities such the Jenolan Limestone Scrub Woodland, and the swamp communities (Carey 2007). In addition, the habitat of threatened or possibly threatened species such as *Trachymene scapigera* (Domin) B.L.Burtt, *Acacia clunies-rossiae* Maiden, *Euphrasia scabra* R.Br. and *Lastreopsis hispida* (Sw.) Tindale are at risk. The distribution of *H. androsaemum* is anticipated to expand within the Blue Mountains, where all south-facing slopes in high-rainfall areas are susceptible to invasion, further jeopardising the conservation status of native communities and species.

Conventional control methods cannot contain or suppress rust-resistant invasions of *H. androsaemum* in New South Wales due to the extent and severity of its distribution, and the difficult terrain it which it grows. Biological control is now the only option available and selection of new pathogens and other agents continues in New Zealand (e.g. New Zealand Landcare Research 2016). Attempts to locate an *avr* gene in tutsan rust have so far been unsuccessful, but *avr* genes have been found in *M. lini*, which infects cultivated flax (Dodds *et al.* 2007) and could guide the search in *M. hypericorum*.

In answer to the question we pose in the title, Australia does need to be concerned about the unabated spread of *H. androsaemum*, and further investment in its biological control is warranted.

REFERENCES
Adair, R.J. (2014). Tutsan rust compatibility evaluation for NSW populations of *Hypericum androsaemum*. Unpublished report to Hawksbury-Nepean Catchment Management Authority.

Bruzzese, E. and I.G. Pascoe (1992). *Melampsora hypericorum*, a rust fungus with potential in the biological control of tutsan, *Hypericum androsaemum*. Proceedings of the International Weed Control Congress, Melbourne, Vic. (Australia), Weed Science Society of Victoria Inc., 1992.

Carey, A. (2007). Protecting swamp communities in the Blue Mountains. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation* 16, 14-16.

Casonato, S. (1998). Biological control of the weed tutsan (*Hypericum androsaemum*) with the rust fungus, *Melampsora hypericorum*. Honours Thesis. Department of Applied Biology and Biotechnology, RMIT, Bundoora.

Casonato, S.G., Lawrie, A.C. and McLaren, D.A. (1999). Biological control of *Hypericum androsaemum* with *Melampsora hypericorum*. Proceedings of the 12th Australian Weeds Conference, eds A.C. Bishop, M. Boersma and C.D. Barnes, pp. 339-42. Tasmanian Weed Society, Hobart, Tasmania.

Department for Environment and Conservation NSW (2006). The vegetation of the Western Blue Mountains. Unpublished report funded by the Hawkesbury. Nepean Catchment Management Authority.

Dodds, P.N., Catanzariti, A.M., Lawrence, G.J. and Ellis, J.G. (2007). Avirulence proteins of rust fungi: penetrating the host-haustorium barrier. *Australian Journal of Agricultural Research* 58, 512-17

Gourlay, H., Rendell, E., Olsen, E., Groetmann, R., Probst, C., Padamsee, M., Bellgard, S. and Goecke, D. (2014). The results of surveys and DNA analysis of tutsan (*Hypericum androsaemum*) and tutsan rust (*Melampsora hypericorum*) in New Zealand and Europe. Proceedings of the XIV International Symposium on Biological Control of Weeds, eds F.A.C. Impson, C.A. Kleinjan and J.H. Hoffman, 2–7 March 2014. Kruger National Park, South Africa.

McLaren, D.A., Bruzzese, E. and Pascoe, I.G. (1997). The potential of fungal pathogens to control *Hypericum* species in Australia. *Plant Protection Quarterly* 12, 81-3.

Nel, T. (2012). How extensive is host resistance in the tutsan–*Melampsora hypericorum* relationship. Honours Thesis. School of Applied Science, RMIT University.

New Zealand Landcare Research (2016). www.landcareresearch.co.nz/publications/newsletters/biological-control-of-weeds/issue-74/tutsan-agents. Cited 17 June 2016.

Parsons, W.T. and Cuthbertson, E.G. (2001). 'Noxious weeds of Victoria', 2nd edition. (CSIRO Publishing, Collingwood).

Williams, K.J., Ford, A., Rosauer, D.F., De Silva, N., Mittermeier, R., Bruce, C., Larsen, F.W. and Margules, C. (2011). Forests of East Australia: the 35th biodiversity hotspot. *In* 'Biodiversity hotspots', pp. 295-310. (Springer).