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RESEARCH ARTICLE

Plant/pathogen interactions observed during host range testing of the rust fungus *Uromyces pencanus*, a classical biological control agent for Chilean needle grass (*Nassella neesiana*) in Australia and New Zealand

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ABSTRACT

Nassella neesiana (Chilean needle grass) is a South American grass species that is a serious weed in Australia and New Zealand. The rust fungus *Uromyces pencanus* is a promising biocontrol agent that could be used to control the weed in both countries. Extensive host range testing has been conducted to explore the specificity of the rust. In this paper we discuss the different degrees of invasion by the rust of the tissues of target and non-target species; the plant defences elicited by such invasion at the cellular level; and their relevance to the biological control of Chilean needle grass.

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Introduction

Nassella neesiana (Trin. & Rupr.) Barkworth (Chilean needle grass, Poaceae) is a perennial tussock-forming grass that is indigenous to South America. In Australia, it is considered: a serious environmental weed (McLaren, Stajsic, & Gardener, 1998); a problem weed of agriculture (Grech, 2007); and, has been declared a Weed of National Significance (WONS) (Thorp & Lynch, 2000). Chilean needle grass was first identified in Australia in 1932 (McLaren et al., 1998) and is now widespread in Victoria (VIC), New South Wales (NSW) and the Australian Capital Territory (ACT), with recent outbreaks occurring in Queensland (Qld), South Australia (SA) and Tasmania (Tas) (Snell, Grech, & Davies, 2007). Chilean needle grass threatens the sheep and wool industries through wool contamination, reductions in animal condition and physical damage from its sharp pointed seeds penetrating the fleece, skins and eyes of livestock (Slay, 2002). In south eastern Australia it also threatens critically endangered native grasslands and is considered as the most significant weed threat to temperate grassland biodiversity (Groves & Whalley, 2002; McLaren et al., 1998).

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Nassella neesiana is also a serious weed in New Zealand (Bourdôt & Hurrell, 1992) where it is classified as an 'unwanted organism' and banned from sale, propagation and/or distribution. Small populations occur in the North Island (near Auckland and in the Hawke's Bay Region) but the worst infestations occur in the Marlborough region, near the top of the South Island. The weed was also found more recently (2008) in North Canterbury in the middle of the South Island.

Both *N. neesiana* and another closely related grass weed, *Nassella trichotoma* (Nees) Hack. ex Arechav. (serrated tussock or nassella tussock), were identified as suitable targets for biological control. Consequently, in 1999 a project was initiated in Argentina (South America) with the aim of finding pathogens with potential to control these weeds. No suitable agents have yet been found for *N. trichotoma* but the rust fungus *Uromyces pencanus* Arth. & Holw. was selected as a promising agent against *N. neesiana* (Anderson, Barton, & McLaren, 2010). This rust has therefore been the object of further studies, particularly experiments to explore its host range. Native and pasture grass species are very important in both Australia and New Zealand and it was therefore necessary to apply the rust to a long list of non-target species in order to test whether or not the rust is host-specific enough to be introduced to these countries. Partial results of the host range testing, with details of microscopic observations of individual species, have been presented elsewhere (Anderson, Gallego, Barton, & McLaren, 2013). In this paper we present the results of further testing performed since then and discuss the results in relation to: the different degrees of invasion of host tissues by the rust; the plant defences elicited by such invasion at the cellular level; and, their relevance to the biological control of Chilean needle grass in Australia and New Zealand.

Material and methods

Host range. Experiment 1

An isolate (Up 27) of *U. pencanus* that originated from a field site in Bahía Blanca, 38°40′S, 62°14′W, Argentina, was selected for host range testing on the basis of its virulence against accessions of *N. neesiana* from seven populations in Australia (Anderson et al., 2010). The list of grass species included in the host range tests is given in Table 1. Their level of relatedness to the target species *N. neesiana* was originally determined by using the phylogenetic trees of Poaceae species published by the Grass Phylogeny Working Group (2001). These levels still remain valid in the light of more recent work on the phylogeny of the genus *Nassella* (Cialdella et al., 2014). Species are listed in order from the most to the least related to the target.

The total number of plants tested per species is given in Table 1, column 2, with batches separated by semicolons. Each species was tested on at least two separate occasions with a few exceptions. Efforts were made to test at least eight individuals per species, but this was not always possible because of low germination rates of available seed and/or poor survival of seedlings. Cases in which this minimum number was not achieved are shown in bold. Efforts were also made to ensure test plants were of a similar age and all tested plants were between four and eight months old at the time of testing.

Inoculum was prepared with urediniospores of the rust. Their viability was assessed prior to each inoculation test by plating a sample of spores on water agar and checking that they

Table 1. Grass species tested for susceptibility to the rust *U. pencanus* isolate 27 listed in order from the most to the less related to the target *N. neesiana*.

Tested species	No. of diseased plants/No. of tested plants per batch	No. of diseased control plants/No. of tested control plants per batch	Macroscopic Symptoms and signs	Development group
Target				
Nassella neesiana [Bacchus Marsh, Vic Au]	1/4; 0/4; 1/4	4/4; 2/4; 3/4	Pustules [12.5%]	Not examined
Nassella neesiana [Ballarat, Vic Au]	0/4; 0/4; 0/4	4/4; 2/4; 3/4	None [0%]	Not examined
Nassella neesiana [Clifton Springs, Qld Au]	1/4; 4/4	4/4; 3/4	Pustules [62.5%]	**6.** Sporulation
Nassella neesiana [Fitzroy flats, NSW Au]	4/4; 4/4	4/4; 3/4	Pustules [100%]	**6.** Sporulation
Nassella neesiana [Goulburn, NSW Au]	**4/4**	4/4	Pustules [100%]	Not examined
Nassella neesiana [Kangarilla, SA Au]	2/2; 3/3; 4/4	1/2; 1/2; 2/4	Pustules [100%]	Not examined
Nassella neesiana [Laverton, Vic Au]	2/4; 2/4; 0/4	4/4; 2/4; 3/4	Pustules [33,33%]	Not examined
Nassella neesiana [Rose Bay, Tas Au]	0/4; 0/8; 0/4	1/2; 1/2; 2/4	Yellow leaf spots [16.67%]	**2.** Penetration
Nassella neesiana [Thomastown, Vic Au]	2/4; 2/4; 2/4	4/4; 2/4; 3/4	Pustules [50%]	Not examined
Nassella neesiana [Truganina, Vic Au]	2/4; 2/4; 3/4	2/4; 4/4; 2/4;	Pustules [62.5%]	Not examined
Nassella neesiana [Auckland, NZ]	0/4; 0/2; 0/4; 0/3	4/4; 2/4; 4/4; 4/5	None [0%]	**2.** Penetration
Nassella neesiana [Hawke's Bay, NZ]	0/4; 0/4; 0/4	4/4; 2/4; 4/4	None [0%]	**2.** Penetration
Nassella neesiana [Marlborough, NZ]	4/4; 4/4	4/4; 3/4	Pustules [100%]	**6**. Sporulation
Level of relatedness 1				
Nassella charruana (Arech.) M.E. Barkworth	0/4; 0/4	1/2; 1/2	None [0%]	**3.** Penetration
Nassella hyalina (Nees) M.E. Barkworth	0/4; 0/4; 0/4	4/4; 2/4; 4/4	Yellow leaf spots [37.5%]	**1.** No penetration
Nassella leucotricha (Trin. & Rupr.) R.W.Pohl	0/2; 0/6	3/4; 2/4	Brown leaf spots [25%]	**3.** Penetration
Nassella tenuissima (Trin.) Barkworth	0/4; 0/4	3/4; 3/4	None [0%]	**1**. No penetration
Nassella trichotoma (Nees) Hack. ex Arechav. [Dalgety, NSW Au]	0/4; 0/4	3/4; 1/4	Yellow leaf spots [62.5%]	**1**. No penetration
Nassella trichotoma [North Canterbury, NZ]	0/4; 0/4	4/4; 2/4	None [0%]	**1.** No penetration
Level of relatedness 2				
Amelichloa caudata (Trin.) Arriaga & Barkworth	0/4; 0/4	3/4; 1/4	None [0%]	**2**. Penetration
Level of relatedness 3				
Austrostipa aristiglumis (F.Muell.) S.W.L. Jacobs & J. Everett	0/4; 0/4	2/4; 4/4	None [0%]	**2.** Penetration
Austrostipa bigeniculata (Hughes) S.W.L. Jacobs & J. Everett.	0/4; 0/4	4/4; 3/4	None [0%]	**2.** Penetration
Austrostipa breviglumis (J.M. Black) S.W.L. Jacobs & J. Everett	0/4; 0/4; 0/6	4/4; 3/4; 1/4	Black leaf spots [21.43%]	**4**. Colonisation
Austrostipa compressa (R.Br.) S.W.L. Jacobs & J. Everett	**2/5**	1/2	Pustules [40%]	**5.** Sporulation
Austrostipa elegantissima (Labill.) S.W.L. Jacobs & J. Everett	**0/4; 0/1**	4/4; 1/4	Black leaf spots [60%]	**3.** Penetration

(*Continued*)

Table 1. Continued.

Tested species	No. of diseased plants/No. of tested plants per batch	No. of diseased control plants/No. of tested control plants per batch	Macroscopic Symptoms and signs	Development group
Austrostipa eremophila (Reader) S.W.L. Jacobs & J. Everett	0/4; 0/2; 0/3	4/4; 1/4; 1/4	Black leaf spots [22.22%]	**3.** Penetration
Austrostipa flavescens (Labill.) S.W.L. Jacobs & J. Everett	0/1; 0/7	2/3; 3/4	Black leaf spots [12.5%]	**3**. Penetration
Austrostipa macalpinei (Reader) S.W.L. Jacobs & J. Everett	**1/2; 1/2**	1/2; 0/2	Pustules [50%]	**5.** Sporulation
Austrostipa mollis (R.Br.) S.W.L. Jacobs & J. Everett	0/1; 0/3; 0/4; 0/4	2/3; 1/4; 3/4; 2/4	None [0%]	**4.** Colonisation
Austrostipa nitida (Summerh. & C.E. Hubb.) S.W.L. Jacobs & J. Everett	**0/2; 0/1/; 0/1**	3/4; 1/4; 2/4	Brown leaf spots [50%]	**4**. Colonisation
Austrostipa nullanulla (J. Everett & S.W.L. Jacobs) S.W.L. Jacobs & J. Everett	0/8	3/4	Black leaf spots [50%]	**4.** Colonisation
Austrostipa platychaeta (Hughes) S.W.L. Jacobs & J. Everett	0/3; 0/2; 0/4	2/2; 1/2; 2/4	Black leaf spots [14.29%]	**4.** Colonisation
Austrostipa rudis (Spreng.) S.W.L. Jacobs & J. Everett	**0/3; 0/3**	2/2; 1/2	None [0%]	**3.** Penetration
Austrostipa scabra (Lindley) S.W.L. Jacobs & J. Everett	0/4; 0/4	1/4; 4/4	None [0%]	**2**. Penetration
Austrostipa setacea (R.Br.) S.W.L. Jacobs & J. Everett.	**0/3; 0/2**	3/4; 3/4	Yellow leaf spots [20%]	**3**. Penetration
Austrostipa stipoides (Hook. f.) S.W.L. Jacobs & J. Everett	0/3; 0/8	2/2; 2/4	Brown leaf spots [100%]	**3**. Penetration
Austrostipa stuposa (Hughes) S.W.L. Jacobs & J. Everett	0/4; 0/4; 0/5	1/2; 1/2; 2/4	Black leaf spots [15.38%]	**3**. Penetration
Austrostipa tuckeri (F.Muell.) S.W.L. Jacobs & J. Everett	**0/4; 0/3**	4/4; 3/4	None [0%]	**2.** Penetration
Austrostipa verticillata (Nees ex Spreng.) S.W.L. Jacobs & J. Everett	**0/2**	1/4	Brown leaf spots [50%]	**4**. Colonisation
Level of relatedness 4				
Piptochaetium napostaense (Speg.) Hack	0/3; 0/4	3/4; 3/4	Yellow leaf spots [25%]	**2.** Penetration
Piptatherum miliaceum (L.) Coss	0/3; 0/2; 0/4; 0/6	2/3; 4/4; 1/4; 3/4	Yellow leaf spots [11.11%], 'blisters' [22.22%]	**4.** Colonisation
Level of relatedness 5				
Avena sativa L.	0/4; 0/4; 0/4	3/3; 3/4; 4/4	None [0%]	**1.** No penetration
Lachnagrostis filiformis (Forst.)Trin (Syn. *Agrostis avenacea* J.F.Gmel.)	0/4; 0/4	2/2; 2/2	None [0%]	**2**. Penetration
Brachypodium distachyon (L.) P. Beauv.	0/4; 0/4	1/4; 2/3	None [0%]	**3**. Penetration
Bromus catharticus Vahl.	0/4; 0/4	3/3; 3/3	Yellow leaf spots [62.5%]	**2**. Penetration
Dichanthium aristatum (Poir.) C. E. Hubbard	0/4; 0/4	2/4; 2/4	None [0%]	**2.** Penetration
Elymus scabrifolius (Döll) J.H. Hunz.	0/4; 0/4	2/4; 3/4	Yellow leaf spots [62.5%]	**2**. Penetration
Eragrostis curvula (Schrader) Nees	0/4; 0/4	4/4; 4/4	None [0%]	**1.** No penetration
Festuca arundinacea Schreb.	0/4; 0/4	3/3; 3/4	None [0%]	**2.** Penetration
Hordeum vulgare Linn.	0/4; 0/4	3/3; 3/3	Yellow leaf spots [62.5%]	**2.** Penetration
Lolium perenne Linn.	0/4; 0/4	3/3; 3/4	None [0%]	**2**. Penetration

(*Continued*)

Table 1. Continued.

Tested species	No. of diseased plants/No. of tested plants per batch	No. of diseased control plants/No. of tested control plants per batch	Macroscopic Symptoms and signs	Development group
Paspalum dilatatum Poir.	0/4; 0/4	2/4: 3/4	Yellow leaf spots [12.5%]	**2.** Penetration
Phalaris aquatica L.	0/4; 0/4	3/3; 3/4	Yellow leaf spots [25%]	**2**. Penetration
Poa ligularis Nees ex Steud.	0/4; 0/4	2/4: 3/4	None [0%]	**2.** Penetration
Secale cereale L.	0/4; 0/4	3/3; 3/4	None [0%]	**2.** Penetration
Triticum aestivum L. cv. ACA 303	0/4; 0/4	3/4; 4/4	None [0%]	**2.** Penetration
Triticum aestivum L. cv. Buck Arriero	0/4; 0/4	3/4; 4/4	None [0%]	**2.** Penetration
Triticum aestivum L. cv. Buck Guapo	0/4; 0/4	3/4; 4/4	Yellow leaf spots [37.5%]	**2**. Penetration
Triticum aestivum L. cv. Coop. Liquén	0/4; 0/4	3/4; 4/4	Yellow leaf spots [37.5%]	**2**. Penetration
Triticum aestivum L. cv. Buck Malevo	0/4; 0/4	3/4; 4/4	Yellow leaf spots [25%]	**2.** Penetration
Triticum aestivum L. cv. Buck Sureño	0/4; 0/4	3/4; 4/4	None [0%]	**2.** Penetration
Triticum aestivum L. cv. Unknown	0/4; 0/4	3/3; 3/3	Yellow leaf spots [37.5%]	**1.** No penetration
Level of relatedness 6				
Ehrharta calycina Sm.	0/4; 0/6	2/2; 2/4	None [0%]	**3.** Penetration
Microlaena stipoides (Labill.) R. Br.	**0/2**	3/4	Brown leaf spots [50%]	**2.** Penetration
Oryza sativa L.	0/4; 0/4	3/4; 3/4	None [0%]	**2.** Penetration
Phyllostachys aurea Carrière ex Rivière and C. Rivière.	**0/4**	4/4	None [0%]	**1.** No penetration
Level of relatedness 7				
Phragmites australis (Cav.) Trin. ex Steud.	0/4; 0/4	3/4; 3/4	None [0%]	**1.** No penetration
Chloris gayana Kunth	0/4; 0/4	3/4; 1/4	None [0%]	**1.** No penetration
Cynodon dactylon (L.) Pers.	0/4; 0/4	1/4; 2/4	None [0%]	**1.** No penetration
Sporobolus rigens (Trin.) E. Desv.	0/4; 0/4	2/4; 2/4	None [0%]	**1.** No penetration
Austrodanthonia geniculata (J.M. Black) H.P .Linder	0/2; 0/1; 0/6	3/4; 1/4; 2/4	None [0%]	**2.** Penetration
Aristida pallens Cav.	0/4; 0/4	3/4; 1/4	Yellow leaf spots [25%]	**1.** No penetration
Bothriochloa springfieldii (Gould) Parodi	0/4; 0/4	2/4; 2/4	None [0%]	**2.** Penetration
Cymbopogon citratus (DC) Stapf.	0/4; 0/3; 0/4	2/4; 1/4; 2/2	None [0%]	**2.** Penetration
Pennisetum clandestinum Hochst. ex Chiov.	0/4; 0/4	1/4; 2/4	None [0%]	**2.** Penetration
Sorghum halepense (L.) Pers.	0/4; 0/4	3/3; 3/4	None [0%]	**2.** Penetration
Themeda triandra Forssk.	0/4; 0/4	1/2; 1/2	None [0%]	**1**. No penetration
Zea mays L.	0/4; 0/4; 0/4	3/3; 3/3; 4/4	None [0%]	**2**. Penetration

Notes: The type of macroscopic symptoms and signs that developed, the percentage of inoculated plants that showed them, and, the group to which each species belongs in regard to the degree of development of the rust within its foliar tissues (Table 3) are given.

Species **in bold**: Those for which less than eight plants were tested. Underlined species: those tested in batches in which half or less of the positive control plants became diseased. Batches separated by semicolons.

germinated. Dry viable urediniospores mixed in talcum powder (ratio 1:30) were applied with a fine paintbrush (10 strokes per leaf) onto the adaxial side of two leaf blades per plant for a maximum of 20 cm per leaf. Alternatively, for species with smaller leaves, the spore-talc mix was applied to more leaves to add up to a total inoculated leaf length of

40 cm per plant, with one stroke of the paintbrush to inoculate each 2 cm section, so that the width of each inoculated section was always that of the paintbrush. Care was taken to try to apply the same quantity of inoculum to similar areas of leaf tissue of each plant to allow for comparison of results. The use of the talcum powder allowed checking for an even distribution of the inoculum. Whenever possible, leaves with an intermediate position in the plants (i.e. not the youngest or the oldest) were selected for inoculation. Inoculated leaves were later sprayed with water. Plants of *N. neesiana* from the ACT were included in each test as positive controls; the number of plants per batch (replicate) is given in Table 1, column 3. Inoculated plants were maintained at 18–20°C under a 12 h (D:L) photoperiod and 100% relative humidity (RH) for 48 h, after which they were kept under the same conditions, but at 70% RH for four weeks, twice as long as the latent period on the *N. neesiana* plants included as positive controls. Note that all susceptible individuals of *N. neesiana* consistently developed signs of disease within 15 days of inoculation both in these studies, and in previous experiments. All inoculated plants were inspected for external symptoms of disease (macrosymptoms) at the end of the experiment.

Susceptibility assessment for Australasian accessions of N. neesiana

Plants belonging to three accessions of *N. neesiana* from New Zealand and 11 from Australia (including those from the ACT used as positive controls) were included in the host range tests (Table 1). The number of uredinia that had developed on each test plant was counted and then a standardised figure was created for each accession by dividing the total number of uredinia for that accession by the area inoculated to give the number of uredinia developing per 10 cm section of leaf blade. This was done for each inoculation replicate. Finally, the average figure of all replicates for each accession was calculated. Each *N. neesiana* accession was then assigned to one of four categories accordingly:

(1) *Not susceptible* when no uredinia developed on any of the tested plants.
(2) *Mildly susceptible*: when on average 1–5 uredinia developed per 10 cm of leaf blade.
(3) *Susceptible*: when on average 6–50 uredinia developed per 10 cm of leaf blade.
(4) *Highly susceptible*: when on average more than 50 uredinia developed per 10 cm of leaf blade.

Plant–pathogen interactions at the cellular level

A week after inoculation, and then at the conclusion of the experiment four weeks after inoculation, samples were randomly taken from two or three leaves per non-target species so that the tissue could be examined microscopically. These samples were cleared and stained using a modification of the Bruzzese and Hasan (1983) method (Flemmer, Anderson, Hansen, & McLaren, 2010), and inspected under the microscope to study the infection process and host reactions to it.

Further study on the non-target species that developed pustules

A small number of uredinia developed on the non-target species *Austrostipa compressa* and *Austrostipa macalpinei.* The length of these pustules, and both the length and

width of urediniospores recovered from them, were measured under a stereomicroscope and microscope respectively with the aid of an eyepiece graticule. This made it possible to compare their size with that of the pustules and spores formed on *N. neesiana*. The existence of statistical differences in the length of pustules and the length and width of spores recovered from the different host species was investigated through nonparametric ANOVAs (Kruskal–Wallis) and *a posteriori* Dunn's all-pairwise comparisons tests.

Experiment 2

A second inoculation experiment was conducted to further investigate the interaction between the rust and plants of *A. compressa* and *A. macalpinei*. All available plants of these two species (four and three respectively) were inoculated as explained above for Experiment 1 but using a higher concentration of spores in talcum powder (1:10).

A sub-sample of the urediniospores recovered from *A. macalpinei* was plated on water agar to test their ability to germinate and another sub-sample was inoculated onto two plants of *N. neesiana* from the ACT using the same methods as in Experiment 1.

Results

Host range

Results for all tested species in respect to the development of macroscopic symptoms are presented in Table 1, column 4 and a few examples are depicted in Figure 1. Nine out of the 11 accessions of *N. neesiana* from Australia that were tested, and one out of the three accessions from New Zealand, were found to be susceptible to *U. pencanus* isolate 27 and developed uredinia (Figure 1(a)). Plants from Ballarat (Victoria) in Australia, and Auckland and Hawke's Bay in New Zealand, did not develop any pustules or macrosymptoms. *N. neesiana* plants from Rose Bay (Tasmania) did not develop pustules but 20% of them did show chlorotic leaf spots. In addition, the rust was able to complete its life cycle and produce pustules on plants of two non-target species: *A. compressa* and *A. macalpinei* (Figure 1(b)).

Susceptibility assessment for accessions of N. neesiana

The number of uredinia that developed on test plants of the target weed varied considerably amongst the 14 different accessions tested. Their assessment scores ranged from 'not susceptible' to 'highly susceptible' (Table 2).

Plant–pathogen interactions at the cellular level

Plant species have been grouped according to the degree of development of the rust within leaf tissues (Table 3). In *Group 1* are those species in which no penetration was observed. *Group 2* comprises those species in which there was rust penetration with the formation of normal substomatal vesicles and penetration hyphae, with no further development. *Group 3* contains those species in which development of the rust continued to the formation of

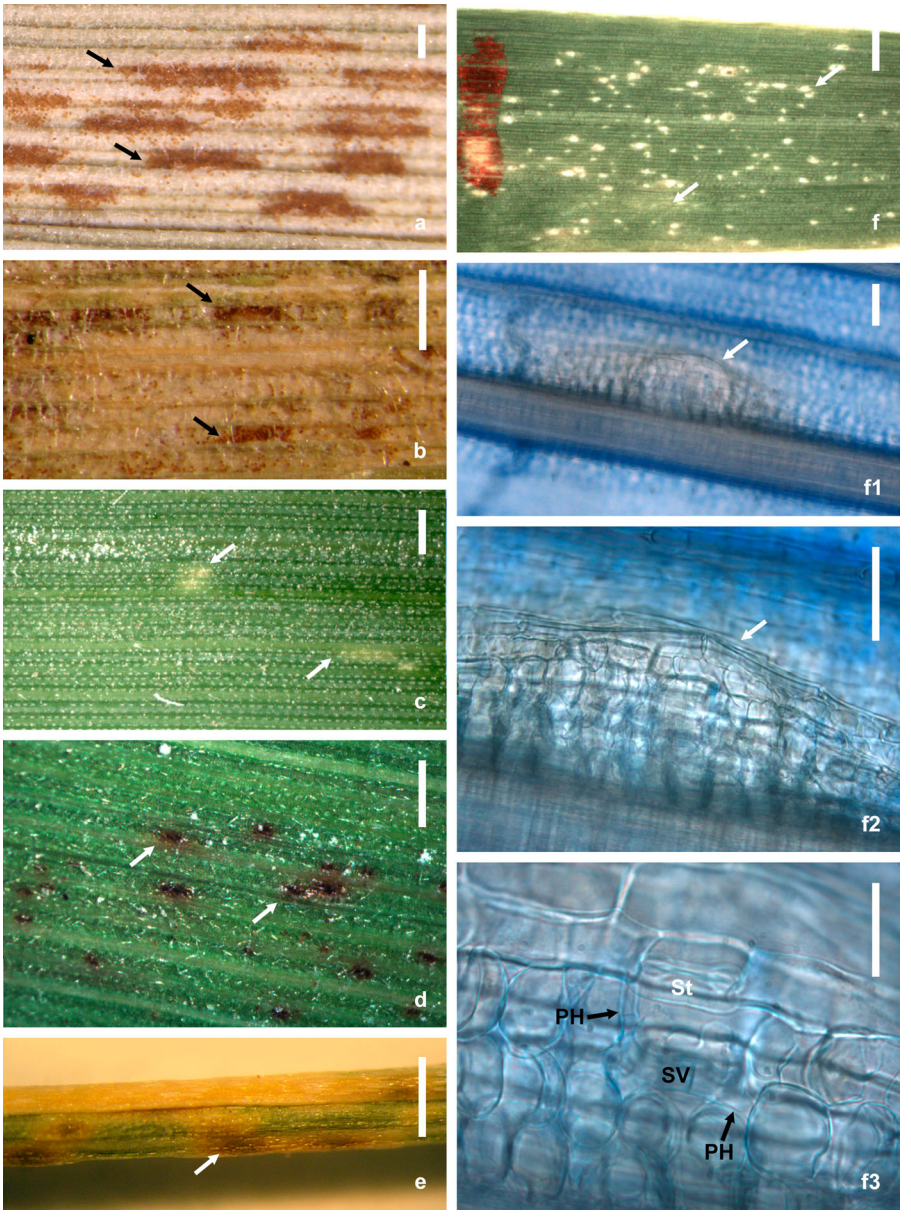

Figure 1. Macroscopic symptoms (white arrows) and signs (black arrows). (a) *N. neesiana*: pustules, scale bar: 0.2 mm; (b) *Austrostipa macalpinei*: pustules, scale bar: 0.2 mm; (c) *Triticum aestivum*: leaf spots, scale bar: 1 mm; (d) *A. elegantissima*: leaf spots, scale bar: 1 mm; (e) *A. stipoides:* leaf spots, scale bar: 1 mm; (f) *P. miliaceum*: blisters, scale bar: 1 mm, f1–f3. Microscopic details of blister tissues, f1–f2 scale bar: 50 µm, f3 scale bar: 20 µm. St: stoma, PH: penetration hyphae, SV: substomatal vesicle.

haustorium mother cells and even a few haustoria, with no further development. *Group 4* contains those species in which the rust progressed further to form small intercellular hyphal networks at some infection sites. *Group 5* contains the two species in which extensive hyphal networks and small spore pustules were formed. Finally, *Group 6* contains the accessions of the target weed that were susceptible to the rust and showed a fully

Table 2. Susceptibility of accessions of *N. neesiana* to the rust *U. pencanus*.

Accession	Highly susceptible	Susceptible	Mildly susceptible	Not susceptible
Australia				
ACT (positive controls)	x			
Bacchus Marsh, Vic			x	
Ballarat, Vic				x
Clifton Springs, Qld		x		
Fitzroy flats, NSW	x			
Goulburn, NSW	x			
Kangarilla, SA	x			
Laverton, VIC		x		
Rose Bay, Tas				x
Thomastown, VIC		x		
Truganina, VIC		x		
New Zealand				
Auckland				x
Hawke's Bay				x
Marlborough	X			

Note: Highly susceptible: > 50 uredinia, susceptible: 6–50 uredinia, mildly susceptible: 1–5 uredinia, not Susceptible: 0 uredinia. Uredinia counted on 10 cm of inoculated leaf blade.

compatible reaction at the cellular level. The defence mechanisms and host reactions identified in the members of each group are also given in Table 3 and some examples depicted in Figures 2 and 3. Within each group, more than one of the described defence mechanisms could be elicited at one time in any one species. A few examples are given:

Group 1. Example = *Themeda triandra*: Normal and abnormal spore germination was recorded. Normal stomatic appresoria were infrequent. In general, appresoria were either non-stomatic or had strange shapes when placed over stomata. On many occasions germ tubes were observed to grow over stomata without recognition (Figure 2(a) and (b)). No penetration was observed.

Group 2. Example = *Austrostipa tuckeri*: In general, spores did not adhere to leaf surfaces and were mostly washed off. Among those that remained, some germinated normally and others abnormally. Both normal stomatic and non-stomatic appresoria were formed in similar numbers. Penetration of the leaf was observed but generally growth stopped shortly after penetration because of thickening of host cell walls upon hyphal contact.

Group 3. Example 1 = *Ehrharta calycina*: Frequent normal spore germination and frequent formation of normal appresoria were observed, but abnormal spore germination and abnormal appresoria were also recorded, albeit less frequently. Penetration through stomata was frequent, as were host reactions to such penetration: cell wall thickening (most frequent); cell collapse; and, cell necrosis. At some penetration sites all of the surrounding cells showed wall thickening (Figure 2(g)). Stomatic cells showed necrosis at penetration sites (Figure 2(h)). A few haustorium mother cells were observed.

Example 2 = *Austrostipa stuposa*: Necrotic leaf spots were formed on two of the tested plants. Inspection under the microscope revealed frequent normal spore germination and frequent formation of normal appresoria, but abnormal germination was also quite frequent. Host cell wall thickening in response to rust invasion was very common. At some penetration sites, all cells in the vicinity were observed to react with cell wall thickening. In these regions there was also host cell necrosis and host cell collapse (less frequent). A few normal haustoria were recorded.

Table 3. Assignment of the tested species to groups in regard to the degree of development of the rust within foliar tissues.

Plant species	Defence mechanisms/nonhost reactions
Group 1: No penetration	
Aristida pallens, Avena sativa, Chloris gayana, Cynodon dactylon, Eragrostis curvula, Nassella hyalina, N. tenuissima, N. trichotoma, Phragmites australis, Phyllostachys aurea, Sporobolus rigens, Themeda triandra.	Normal appresoria without penetration; abnormal germination; non-stomatic appresoria; no recognition of stomata by germ tubes.
Penetration	
Group 2: Rust penetration occurs but growth stops shortly after	
Amelichloa caudata, Austrodanthonia geniculata, Austrostipa aristiglumis, A. bigeniculata, A. scabra, A. tuckeri, Bromus catharticus, Bothriochloa springfieldii, Cympopogon citratus, Dichanthium aristatum, Elymus scabrifolius, Festuca arundinacea, Hordeum vulgare, Lachnagrostis filiformis, Lolium perenne, Microlaena stipoides, Oryza sativa, Paspalum dilatatum, Pennisetum clandestinum, Phalaris aquatica, Piptochaetium napostaense, Poa ligularis, Secale cereale, Sorghum halepense, Triticum aestivum, Zea mays.	Low adherence of spores to leaf surface; abnormal spore germination; non-stomatic appresoria; normal appresoria without penetration or with substomatal vesicle and penetration hyphae with no further growth. If growth continued to contact host cells, these showing wall thickening and/or necrosis and /or collapse, and/or granulation.
Group 3: Haustorium mother cells are formed, with or without formation of haustoria, causing host reaction and growth cessation	
Austrostipa elegantissima, A. eremophila, A. flavescens, A. rudis, A. setacea, A. stipoides, A. stuposa, Brachypodium distachyon, Erhartha calycina, Nassella charruana, N. leucotricha.	Abnormal spore germination, abnormal appresoria, non-stomatic appresoria; at some penetration sites necrosis of stomatic cells and thickening of walls of all surrounding cells, in others surrounding cells showing disorganisation of contents and/or necrosis, host cells bearing haustoria collapsed and/or showing necrosis, encased and/or collapsed haustoria frequent.
Colonisation	
Group 4: Haustoria are formed allowing for some intercellular mycelium development	
Austrostipa breviglumis, A. mollis, A. nitida, A. nullanulla, A. platychaeta, A. verticillata, Piptatherum miliaceum	Abnormal spore germination, necrosis of stomatic cells at penetration sites, encased and/or collapsed haustoria, necrosis of host cells bearing normal haustoria, plasmolysis of intercellular hyphae.
Sporulation	
Group 5: Haustoria, well-developed mycelia and pustules are formed	
Austrostipa compressa, A. macalpinei	Necrosis, and/or collapse of host cells at infection sites, thickening of host cell walls upon contact by hyphae, encased haustoria.
Group 6: Fully compatible host reaction	
Nassella neesiana (accessions from the ACT, Clifton Springs and Fitz Roy Flats in Australia and from Marlborough in New Zealand)	None

Note: Resistance reactions found at the cellular level in members of each group are shown.

Group 4. Example 1 = *Austrostipa nullanulla*: On this species there was frequent normal spore germination with frequent formation of normal appresoria and subsequent penetration in the leaves. The cells of many stomata at infection sites became necrotic. Growth usually stopped upon contact with host cells, where there frequently was thickening of walls at points of contact, but some formation of both normal and encased haustoria (Figure 3(a)) was recorded. At certain infection sites the development of small hyphal networks could be observed. Host cell wall thickening, cell collapse and cell necrosis in the vicinity of such networks were also recorded.

Example 2 = *Austrostipa verticillata*: Necrotic spots developed on one of the two plants tested. Inspection under the microscope revealed normal spore germination, formation of

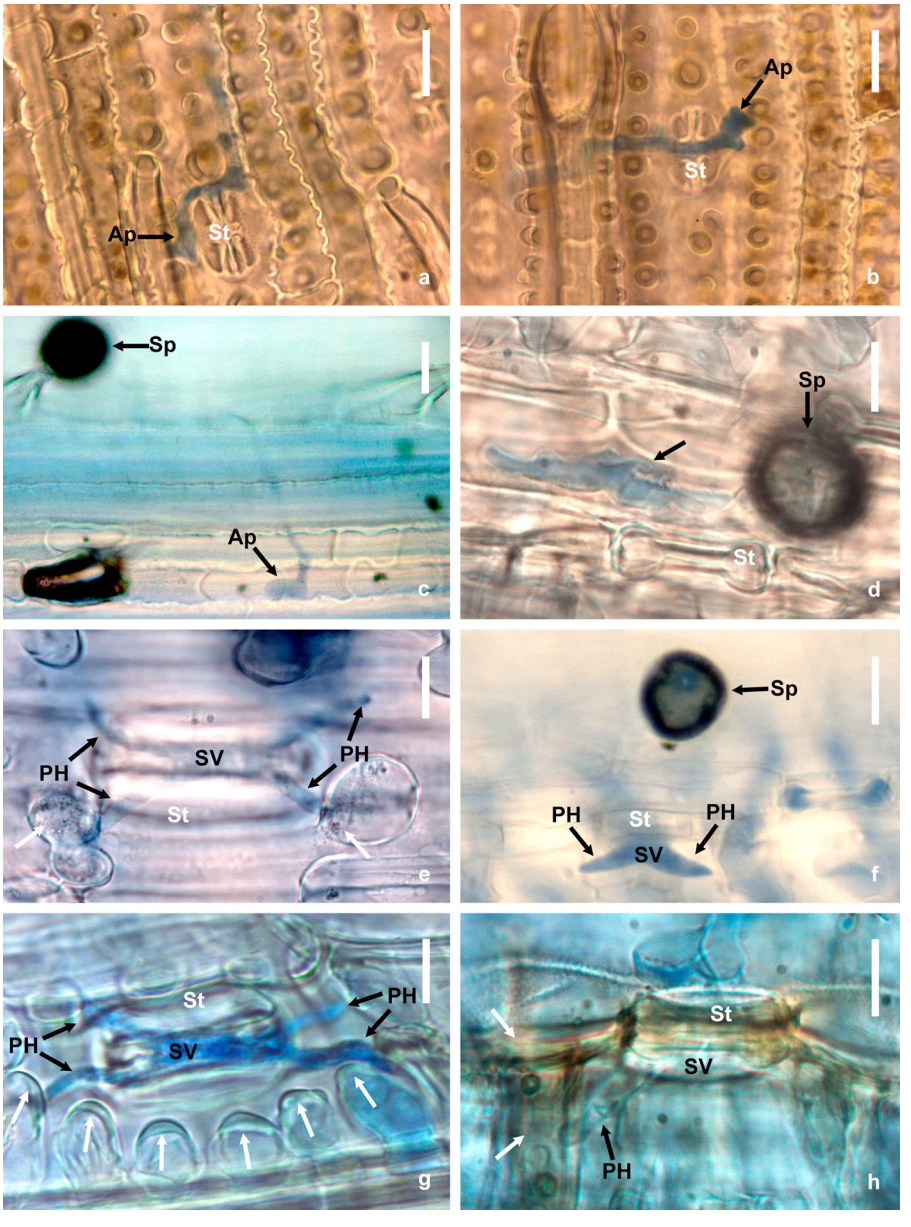

Figure 2. Microscopic observations. (a) and (b) *T. triandra*: nonrecognition of stomata, non-stomatic appresoria (arrows), scale bar: 15 µm; (c) *Phragmites australis*: non-stomatic appresorium (arrow), scale bar: 20 µm; (d) *Austrodanthonia geniculata*: abnormal spore germination (arrow), scale bar: 15 µm; (e) *Secale cereale*: 'granulation' in cells contacted by penetration hyphae, scale bar: 15 µm; (f) *Lolium perenne*: growth stop shortly after penetration, scale bar: 20 µm; (g) *E. calycina*: thickening of host cell walls at penetration site (white arrows), scale bar: 10 µm, (h) *E. calycina*: necrosis of host cells at penetration site (white arrows), scale bar: 15 µm. Ap: appresorium; PH: penetration hyphae; Sp: spore; St: stoma; SV: substomatal vesicle. Fungal features in black; host features and reaction in white.

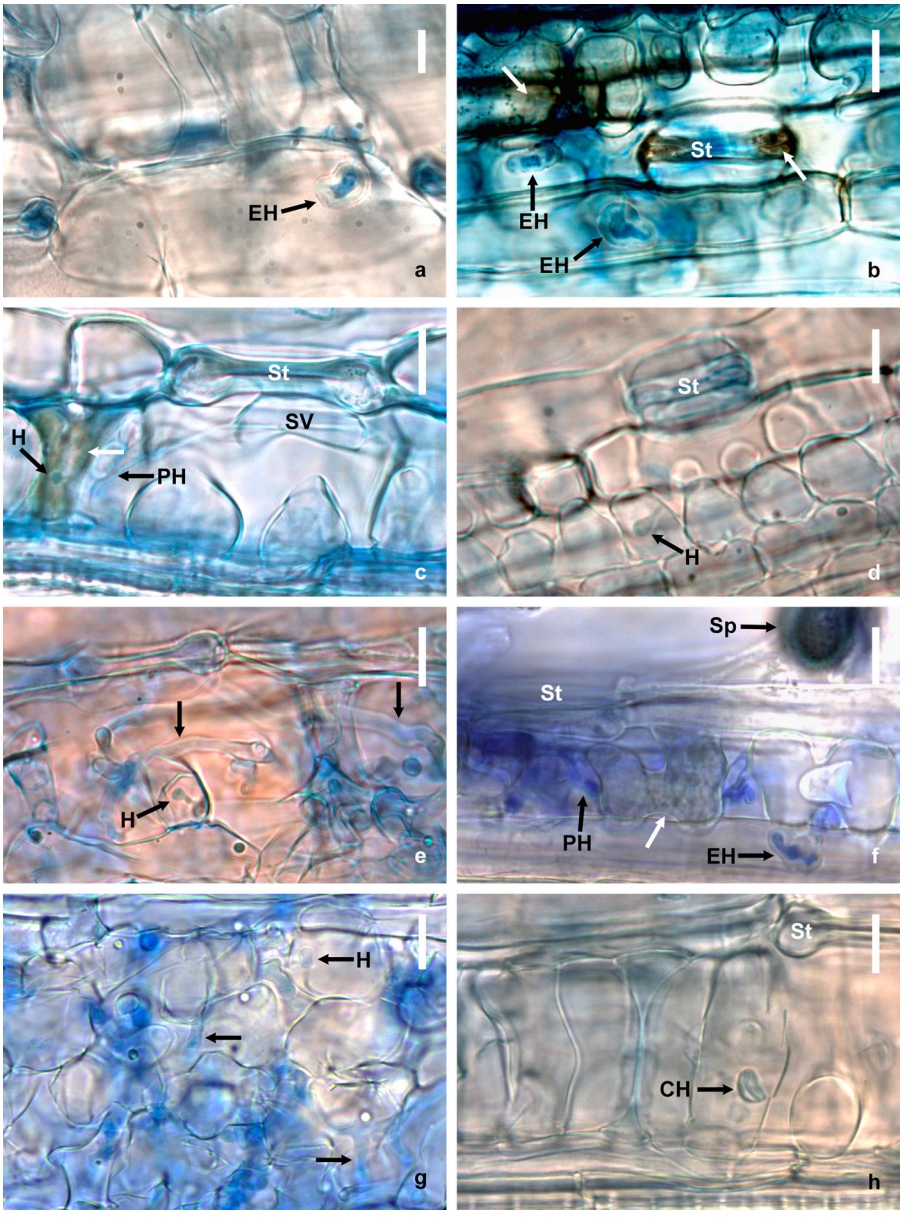

Figure 3. Microscopic observations. Haustoria. (a) *Austrostipa nullanulla:* encased haustorium, scale bar: 10 µm; (b) *A. breviglumis*: encased haustoria (black arrows) and cell necrosis (white arrow), scale bar: 10 µm, (c) *A. mollis*: normal haustorium (black arrow) in necrotic cell (white arrow), scale bar: 10 µm; (d) *P. miliaceum*: normal haustorium (black arrow), scale bar: 10 µm; (e) *A. compressa*: normal haustorium and mycelium (black arrows), scale bar: 10 µm; (f) *A. compressa* (plant that did not develop pustules): encased haustorium (black arrow) and cell necrosis and granulation (white arrow), scale bar: 10 µm; (g) *A. macalpinei*: normal haustorium and mycelium (black arrows), scale bar: 15 µm; (h) *A. rudis*: collapsed (wine-glass shaped) haustorium (black arrow), scale bar: 10 µm. H: haustorium; CH: collapsed haustorium; EH: encased haustorium; PH: penetration hyphae; Sp: spore; St: stoma; SV: substomatal vesicle. Fungal features in black; host features and reaction in white.

normal appresoria and leaf penetration. Host cell wall thickening and cell necrosis were observed in response to penetration. Some limited mycelial development was observed.

Example 3 = *Piptatherum miliaceum*: Four of the 18 plants tested developed 'blisters', swellings on leaf surfaces that somewhat resembled pustules (Figure 1(f)). Closer observations revealed these were composed of host, rather than rust, tissues. Here both hyperplasia and hypertrophy seem to occur (Figure 1(f1–f3)). Frequent normal spore germination, formation of normal appresoria and leaf penetration were recorded. Development of quite large hyphal networks was observed. Many haustoria were recorded. Some of these were normal (Figure 3(d)), others were encased and one was collapsed (wine-glass shape). Host cell wall thickening, cell collapse and cell necrosis were observed to occur frequently in response to fungal invasion.

Group 5 contains only two species.

(1) *Austrostipa compressa*
Microscopic examination of plants that became infected revealed the presence of a well-developed hyphal network and normal haustoria (Figure 3(e)). Some thickening of host cell walls was observed but this was infrequent. Microscopic examination of plants that did not become infected also showed some development of intercellular mycelia but normal haustoria were less frequent while many were encased (Figure 3(f)). Other defence mechanisms seen to be triggered in these plants were thickening of host cell walls and host cell necrosis. Some sections of fungal mycelium appeared to have thickened walls.

(2) *Austrostipa macalpinei*
Microscopic examination of plants that became infected revealed the presence of a well-developed hyphal network and normal haustoria (Figure 3(g)). Some thickening of host cell walls, cell collapse and necrosis in response to rust invasion was recorded. Microscopic inspection of plants that did not develop spore pustules revealed the presence of the same defence mechanisms as in infected plants and a quite extensive intercellular mycelium.

Further study on test species that developed pustules

Results of the two inoculation experiments performed on *A. compressa* and *A. macalpinei* are given in Table 4. The number of pustules formed on *A. compressa* was at least 50 fold lower than on the controls in Experiment 1 and 27 fold lower in Experiment 2. In the case of *A. macalpinei* the number of pustules was 25 and 10 fold lower respectively. Moreover, significant differences were found in the length of the pustules ($H = 36.77$, $P < .0001$) and in the length ($H = 28.56$, $P < .0001$) and width ($H = 12.51$, $P < .001$) of urediniospores formed on the different host species. The pustules formed on both *Austrostipa* species were found to be significantly shorter ($Z = 15.661$, $P < .001$) than those formed on *N. neesiana* (Figure 4), as were the urediniospores (Figure 5(a)) recovered from such pustules ($Z = 16.701$, $P < .01$). Only the urediniospores from *A. compressa* were found to be significantly narrower ($Z = 16.746$, $P < .01$) than those from *N. neesiana* (Figure 5(b)). Spores recovered from *A. macalpinei* germinated normally on water agar, but were not able to infect either of the two inoculated plants of *N. neesiana* from the ACT.

Table 4. Number of pustules of *U. pencanus* developed on *Austrostipa compressa* and *A. macalpinei* compared with those on the positive controls included in their respective batches.

Species	Experiment	No. of plants with pustules/No. of tested plants	No. of pustules/10 cm leaf blade
Nassella neesiana ACT (positive controls)	1	2/4	441, 303, 0, 0
A. compressa	1	2/5[a]	6, 4, **0**[a]
A. macalpinei	1	1/4[a]	12, **0**[a]
Nassella neesiana ACT (positive controls)	2	4/4	772, 716, 682, 413
A. compressa	2	2/4[a]	15, 12, **0**[a]
A. macalpinei	2	2/3	40, 9, **0**

[a]One or two of the inoculated plants were dead at the end of the experiment (30 days) and could not be evaluated. Mortality was more likely the result of the plant's short life cycle than infection by the rust. Experiment 1 = Spore concentration of the inoculum 1:30, Experiment 2: = Spore concentration of the inoculum 1:10.

Discussion

Host range

The host range of a pathogen is defined by the plant species it can infect and on which it can successfully complete its life cycle (Bettgenhaeuser, Gilbert, Ayliffe, & Moscou, 2014). *U/ pencanus* isolate 27 was applied to: 14 accessions of the target weed *N. neesiana* (one of them used as a positive control); two accessions of another potential target weed (*N. trichotoma*); six wheat cultivars; and, to one accession of each of 57 non-target species. Of these, the rust was able to complete its life cycle and sporulate on 10 accessions of *N. neesiana* and on two non-target *Austrostipa* species: *A. macalpinei* and *A. compressa*.

Note that although for the majority of the tested species, all or most of the control plants became diseased in at least one of the batches in which they were tested, not all of the positive control plants developed disease symptoms during our host range tests. We believe the inconsistency in results with the positive control plants was because of

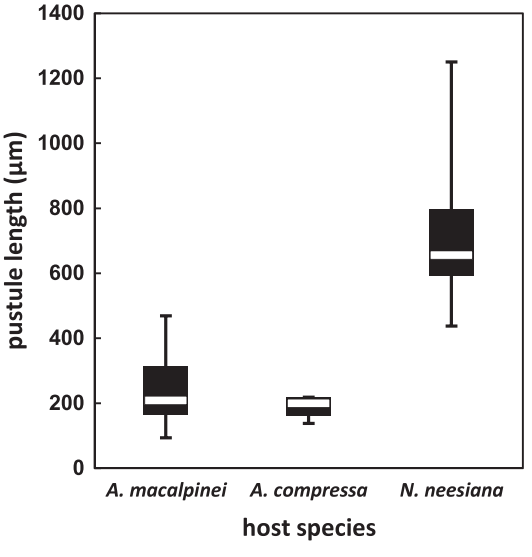

Figure 4. Length of pustules formed on two non-target species, *Austrostipa malcapinei* and *A. compressa*, as compared with those on *N. neesiana*.

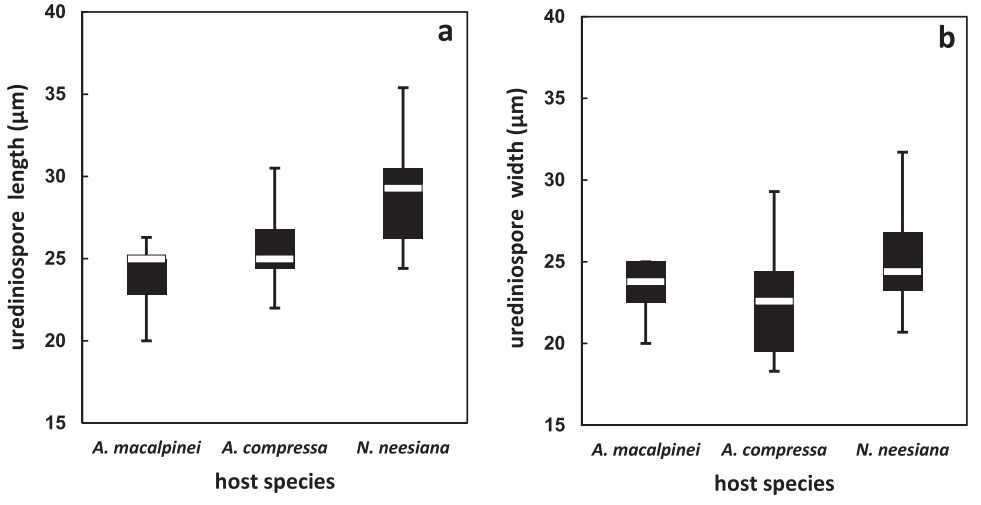

Figure 5. Length (a) and width (b) of urediniospores formed on two non-target species, *A. malcapinei* and *A. compressa*, as compared with those on *N. neesiana*.

variation in genotype and susceptibility between individuals in the accession used as the positive control (ACT), rather than because the inoculum and conditions provided were inappropriate for infection to take place. Proof of this is that all tested plants of fully susceptible accessions of the target weed, such as Kangarilla from Australia and Marlborough from New Zealand, developed disease symptoms, performing better in this respect than the positive controls included in their respective batches. The decision to use plants from the ACT as positive controls was made early in the course of the project, even when it was known that sometimes not all individuals became infected in artificial inoculation trials, because it was the accession on which the rust performed best at the time. Plants from Kangarilla and Marlborough (mentioned above) did not become available until much later. The reason a 1:30 spore concentration in talcum powder was used as inoculum, rather than the 1:10 found to render more consistent results on plants from the ACT (Anderson et al., 2010), was simply that it was not logistically feasible to produce enough spores to prepare a higher concentration of inoculum, plus complete the testing, within the time frame of the study. It is acknowledged that it was stated in an earlier publication (Anderson et al., 2010) that producing urediniospores of *U. pencanus* was relatively easy. However, that remark was made in relation to previous work on *Puccinia nassellae* from *N. trichotoma*. Mass producing *U. pencanus* is indeed easier than mass producing *P. nassellae*, but it soon became evident that the time and resources needed to mass produce Up 27 were in fact much greater than anticipated. This was because: (1) environment cabinet space to culture the rust was limited, (2) not all inoculated plants produced spores (regardless of the spore concentration used as inoculum) and (3) for a great part of the project it was necessary to travel three times a month to a quarantine facility 700 km from where the project was based to grow and test the species exotic to Argentina.

In view of the above, steps were taken to ensure test results would still be reliable and useful. Firstly, several positive control plants were included in each batch so that at least one plant would develop pustules, thus indicating incubation conditions were adequate for

the development of disease. Secondly, comprehensive microscopic studies were undertaken to complement macroscopic observations. There were not many replicates where only half or less of the positive control plants became diseased, but where this did occur the results are not robust enough to be conclusive. The species tested in such replicates are underlined in Table 1. Of the non-target species among these, *Nassella charruana* would not pose a problem should it eventually prove susceptible to the rust because it is a weed. *Dichanthium aristatum*, *Cynodon dactylon, Sporobolus rigens, Bothriochloa springfieldii, Pennisetum clandestinum* and *T. triandra*, all belong to the first and second developmental groups presented in Table 3. Microscopic observations revealed that in members of these groups there was either no penetration of the rust into inoculated leaves, or, that fungal growth stopped shortly after penetration, indicating it is very unlikely there could be disease development in any of these species. *A. stuposa*, belongs in developmental group 3. In that group there was a greater development of the rust within leaf tissues than in those mentioned above, but important host cell reactions did not allow for the development of mycelia. Finally, *A. verticillata*, belongs to developmental group 4, which means some limited mycelium development was recorded. Additional testing would be needed for these two last species (especially since only two individuals of the latter were tested), to confidently define their status as either resistant or susceptible to the rust.

While it is a concern that uredinia were formed on two non-target species, results show that the number of pustules formed on *A. compressa* and *A. macalpinei* was much lower (at least 10 fold) than on the target species and so the rust would be expected to cause less, if any, significant damage on these species. The size of both the pustules and the spores recovered from both *Austrostipa* species was smaller than those on the target weed, further suggesting a somewhat restricted disease development on these species. The fact that spores collected from artificially infected *A. macalpinei* failed to infect *N. neesiana* plants is encouraging but by no means conclusive as there were inadequate numbers of spores available for this experiment. Despite the gathered evidence, more tests on these two *Austrostipa* species are needed to fully assess the risks to which they would be exposed should the rust be introduced in Australia, as it was not possible to perform them during the course of the studies reported on here.

It had been planned to challenge both susceptible *Austrostipa* species to both higher and lower spore concentrations, to test the impact of infection on them. We also hoped to test whether spores recovered from these non-target plants were able to infect new plants of the same species. Unfortunately, and despite many efforts, it was not possible to produce a new set of plants for Experiment 2 or further experimentation. The cultivation of these species in quarantine proved to be extremely difficult: hundreds of seeds had to be germinated to obtain the few plants that were tested. All the seed that was imported into the containment facility for host range testing was used up and it was not possible to get more because the permits necessary to export seed of these species from Australia had expired.

Austrostipa compressa and *A. macalpinei* are fire-ephemeral-species: that is, they are short-lived plants with seeds that persist in the soil and germinate after a fire or physical soil disturbance (Baker, Steadman, Plummer, & Dixon, 2005). As such, many *A. compressa* and *A. macalpinei* plants flowered and died before they could be tested or indeed, during the tests. The very short life cycle of these grasses might provide them with some protection from *U. pencanus* if it were released in Australia. If many of the plants did not survive

long enough in the laboratory for the rust to sporulate on them, then the same may be true in the field; and if the rust cannot sporulate, it will not survive to infect the next generation of these grasses when they germinate after a fire or some other disturbance.

All of the plants in Levels 1–4 of relatedness in Table 1 belong to the same tribe as *Nassella* (the Stipeae). Level 5 represents different tribes in the same subfamily (Pooideae) and levels 6 and 7 belong in different subfamilies of the same family (Poaceae). While the rust could only produce spores on plants that were very closely related to the target weed (in the same tribe: Stipeae), there was not a direct correlation between the degree of invasion of host tissues and the level of relatedness to the preferred host. We expected the rust to progress further in those species most closely related phylogenetically to *N. neesiana*. Instead, a greater development was observed within tissues of several *Austrostipa* species and *P. miliaceum,* than in members of the same genus as the target, such as *N. charruana* and *N. leucotricha*, and even than in some accessions of *N. neesiana*. Nonetheless, the rust was not able to get beyond group 3 (penetration) on plants that were at a level of relatedness of 5 or above. Therefore, these results indicate that the rust is very unlikely to infect any grasses outside of the Stipeae, and only a small subset of those belonging to that tribe.

This type of result (i.e. where host preferences do not strictly follow host phylogenetics) is not common in the biological control literature. In general, pathogens that are host-specific enough to be considered suitable biocontrol agents are very good plant taxonomists (i.e. they are most likely to cause disease on the closest relatives of their main hosts (Barton (née Fröhlich), 2004). However, there have been some previous records (Parker, Holden, & Tomley, 1994; Wood, 2006). Similar findings were reported by Morin, Aveyard, Lidbetter, & Wilson (2012) who found no apparent correlation between the presence or the absence of symptoms of disease caused by the rust *Puccinia psidii* G. Winter, and the phylogenetic relatedness of taxa within the Myrtaceae, although all of the susceptible plants were in the Myrtoideae subfamily.

Plant–pathogen interactions

Plant–pathogen interactions are complex; their outcomes range between full susceptibility (host) to complete immunity (nonhost) but the boundary between both extremes is not always a sharp line. Nonhost resistance reactions to rusts range from those in which the pathogen is physically incapable of infecting the host, passing through different degrees of tissue colonisation without sporulation, to those in which rust pustules are formed but are typically much smaller than those on fully susceptible hosts (Bettgenhaeuser et al., 2014). Such a range of reactions has been encountered during our studies with *U. pencanus* isolate 27. To classify the continuum of possible outcomes Bettgenhaeuser et al. (2014) suggest that in addition to 'host' and 'nonhost' the terms 'Intermediate host' and 'Intermediate nonhost' are required to describe the in-between interactions. The pathogen's inability to penetrate the host and/or to form haustoria would be requirements to consider a plant as a true 'Nonhost'. The majority of the tested species (38 out of 58), belonging to our developmental groups 1 and 2, would fall into this category. A plant in which the rust can form haustoria but is unable to complete its life cycle, or, on which sporulation is very rare, would be an 'Intermediate nonhost'. Such is the case of the 18 tested species placed in our developmental groups 3 and 4. A plant on which

small pustules are frequently formed should be considered as an 'Intermediate host'. *A. compressa* and *A. macalpinei,* in our developmental group 5, could be considered as such.

In almost all tested taxa one or more defence mechanisms were found to occur in response to the pathogen. The exceptions were susceptible accessions of the preferred host, to which the rust is an adapted pathogen. These defence mechanisms generally did not prevent penetration of host tissues, which occurred to some degree in most tested species. No inhibition of spore germination was observed. Spore inhibition was encountered by Evans and Tomley (1994) in a similar study and was attributed to the presence of powerful fungitoxic compounds within plant cuticles. According to Heath (1981) reports on reduced germination in nonhosts are rare. More commonly urediniospores as these tend to germinate well but then germ tubes have difficulties in locating and recognising stomata. In this study the majority of urediniospores germinated normally on most tested species and most of the germ tubes formed located stomata properly. Notwithstanding, there was some degree of abnormal germination with the formation of distorted swollen germ tubes (e.g. on *Austrodanthonia geniculata*).

Appresoria were mostly formed over stomata but abnormally shaped and non-stomatic ones were also recorded on many species. Only on a few, such as *Elymus scabrifolius* and *Poa ligularis*, non-stomatic appresoria were more abundant than stomatic ones. On very few occasions were normal stomatic appresoria recorded with no further development of the rust. Moreover, when there was rust penetration into the substomatal cavity, infection hyphae tended to continue growth until they made contact with host cells, which reacted to contact in some way. This is in accordance with previous findings that suggest infection hyphae seem to grow normally until the time when a haustorium should be initiated (Heath, 1981).

Appositional cell wall formation in plants is characterised by a series of molecular events set in motion by an inducing agent, manifested by the continuing deposition of wall structural materials in a progressively thickening of the cell wall (Sherwood & Vance, 1990). In our studies, deposition of callose-like materials on walls of cells next to infection hyphae was the most common host-defence observed and seemed to prevent the formation of haustoria, precluding further fungal development. Cessation of fungal growth may result from growth inhibitory properties of specific metabolites at the site of appositional wall formation together with, or apart from, the physical barrier in itself (Sherwood & Vance, 1990). In our studies the thickening of cell walls seemed to act mostly as a physical barrier. However, the fact that in some cases all cells in the vicinity of the infecting hyphae reacted with wall thickening, resulting in cessation of fungal growth even without any of the hyphal tips being in contact with the cell walls, would indicate that in those instances some kind of inhibitory substance was operating.

Other reactions, observed in response to the presence and/or contact of infection hyphae were 'cell granulation' (Parker et al., 1994), and cell collapse and/or necrosis. In some cases these reactions appeared to prevent haustoria formation but in others, normal-looking haustoria were observed within collapsed and/or necrotic cells, indicating cell death occurred after, and most likely in reaction to, the formation of haustoria. Sometimes haustoria were surrounded by thick sheaths and became encased and isolated, by depositions of callose-like materials. In some cases encased haustoria collapsed adopting a typical 'wine-glass' shape (Evans & Tomley, 1994).

In general, these reactions prevented or limited the development of intercellular mycelium, but in a few species (group 4) small hyphal networks could be observed at certain infection sites and in two *Austrostipa* species (group 5) there was extensive intercellular mycelium and pustule formation on some individuals. Inspection of samples belonging to these two species, both from individuals that became infected and from others that did not, revealed that several defence mechanisms were elicited. These precluded the development of the rust at certain infection sites while not in others. These observations are consistent with those of Heath (1982) who pointed out that for any given host–pathogen combination, infection sites are rarely identical and that variability is usually more common in resistant hosts than in susceptible ones. Reactions such as thickening of cell walls, cell collapse and encasement of haustoria recorded in *A. compressa* and *A. macalpinei,* were never observed at any of the infection sites in the fully compatible association between *U. pencanus* and *N. neesiana* from the ACT (Flemmer et al., 2010).

Another important observation is that there was not always a correlation between the presence of macrosymptoms and the degree of fungal development. For example, some chlorotic leaf spots were formed on inoculated leaves of *Aristida pallens*, belonging to group 1, where no penetration of host tissues took place, while there were no macrosymptoms on *E. calycina* plants, belonging to group 3, where development proceeded up to the formation of haustorial mother cells, nor on *Austrostipa mollis*, belonging to group 4, in which a few normal haustoria were recorded and some of the invaded cells became necrotic. When necrotic leaf spots were formed, their colour varied ranging from light brown to black. According to Heath (1982) difference in colour reflects the way in which the host cells died. Some of the inoculated plants of *P. miliaceum* developed white 'blisters' that somewhat resembled pustules. When inspected microscopically these were formed mostly by host, rather than fungal, tissues. The occurrence of similar macrosymptoms has been reported previously (Evans & Tomley, 1994).

If it were to be released, *U. pencanus* isolate 27 (Up 27) should cause severe damage to most of the *N. neesiana* populations in Australia, and the most serious (Marlborough) infestation in New Zealand. However, in both countries some populations of *N. neesiana* are unlikely to be damaged by Up 27 and it is possible that further isolates of *U. pencanus* may be required. If further *U. pencanus* isolates are found in Argentina it can be expected that their host range with respect to non-target species will be similar to that of Up 27. Indeed, they are likely to have narrower ranges because Up 27 was selected because it had the broadest intraspecific host range (i.e. attacked *N. neesiana* from the largest number of populations) among the five isolates tested (unpublished results).

The fact that the rust is able to sporulate on two species of Australian native grasses is a cause for concern. As mentioned above, these two *Austrostipa* species are fire ephemerals. Such a life cycle would make it unlikely that a continual population of *U. pencanus* could be maintained in the field unless a population of the target plant, *N. neesiana,* was in close association. The distribution of *A. compressa* is restricted to the south west coast of Western Australia while *A. macalpinei* occurs in restricted locations in both Victoria and South Australia. None of these species currently overlaps with current *N. neesiana* distributions but climate matching suggests such overlap could be a possibility in the future

(Bourdôt, Lamoureaux, Watt, Manning, & Kriticos, 2012). The prevailing winds in Australia generally blow from Western Australia towards Eastern Australia (Kalma, Speight, & Wasson, 1988). Given that most *N. neesiana* infestations occur in south eastern Australia it is unlikely but not impossible that airborne spores could reach Western Australian *Austrostipa* populations. If the rust were to become very common on the target weed then it would be possible for humans to inadvertently move spores of the pathogen from east to west.

Neither *A. compressa* nor *A. macalpinei* is regarded as rare or threatened in Australia (Australia's Virtual Herbarium [AVH], 2013) though the latter is considered an uncommon (rare) species in Victoria (V. Stajsic, personal communication). If further experiments were to suggest *U. pencanus* could also complete its life cycle on the two *Austrostipa* species that were inadequately tested here (*A. stuposa* and *A. verticillata*) then their geographic distribution, habit and threatened status should also be assessed. Note that more than 50 exotic *Uromyces* species have been recorded in Australia with no reported negative impacts to indigenous species (Jacky Edwards & Roger Shivas, personal communication).

Authorities approved the introduction and release of *U. pencanus* into New Zealand for control of *N. neesiana* on 22 June 2011 (Environmental Risk Management Authority [ERMA], 2011). Unfortunately, export permits have not yet been granted by the relevant authorities in Argentina. The application to introduce and release *U. pencanus* in Australia has been reviewed and authorities have requested further host range testing. They have asked for assessment of additional wheat varieties grown in Australia, and follow-up testing of the *Austrostipa* species that were identified in this study as requiring further analysis. The authors agree that further research on *U. pencanus* will be valuable to authorities weighing up the costs and benefits of releasing this rust in Australia.

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