The Biology and Host range of the Proposed Biological Control Agent *Uromyces pencanus*.

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1. Summary

- *Nassella neesiana* (Chilean needle grass = CNG) is a significant agricultural and environmental weed in Australia and New Zealand and the two countries have worked together for ca. ten years to find a suitable biological control agent to use against it

- The only suitable agent found in the home range of the weed (South America), and the subject of this application, is the rust fungus *Uromyces pencanus*

- Most of the pathogens that have been used for classical biocontrol of weeds world-wide have been rusts, and they have never caused unpredicted non-target damage in the field

- *Uromyces pencanus* has been observed killing infected leaves, and reducing seed production, of CNG in the field in Argentina. The rust is particularly damaging in dry weather. Laboratory experiments have confirmed the rust can reduce the growth of infected plants

- Rusts have complicated life-cycles that include up to five different types of spores (spores of rusts are like the seeds of plants)

- A rust may complete its life cycle on only one host, or it can form some spores types on one host and other types on another (not closely related) host

- *Uromyces pencanus* has been reported in the literature to form three types of spores on CNG: urediniospores, teliospores and aeciospores. We present evidence that in fact, it only forms urediniospores and teliospores on CNG. The aeciospores often found on CNG belong to the life cycle of another rust (*Puccinia graminella*)

- If *Uromyces pencanus* did produce aeciospores on CNG, then it would unequivocally only have one (main) host. Since it does not, it is possible that it is also has a second (alternate) host. We present evidence that the teliospores of *U. pencanus* have lost the ability to produce basidiospores. If this is true, then the rust is not longer able to use an alternate host.

- Mycoparasites that exist in new Zealand are similar to those that exist in Argentina, so there is no reason to believe they will significantly hamper the activity of the rust here

- It is extremely unlikely that the introduction of *U. pencanus* would lead to adverse impacts on native rusts through hybridization

- Extensive host range testing has been conducted with a single strain of *U. pencanus* (UP 27). It was applied to 65 taxa including 12 populations of CNG, 2 populations of the weed nassella tussock and 7 cultivars of wheat (in total, 47 species were tested). UP 27 was shown to be

highly host specific: it only formed pustules on the target weed, and only on 9 of the 12 CNG populations tested.

- Strain UP 27 will probably only attack CNG from the South Island of New Zealand. Therefore, other strains of *U. pencanus* in Argentina and may be wanted in future to control CNG infestations in the north island. If so, there is no reason to believe these other strains would have a broader host range than UP 27.

- If *Uromyces pencanus* were introduced to New Zealand, there is no reason to believe its host range would broaden over time through evolution

- To conclude: The introduction of *Uromyces pencanus* to New Zealand is unlikely to cause any significant negative impact on native or otherwise valued plants or fungi

2. Introduction

2.1 History of this project

Nassella neesiana (Chilean needle grass (CNG), Poaceae) and *Nassella trichotoma* (serrated tussock) are significant agricultural and environmental weeds in Australia and New Zealand. A biological control program was initiated by the Australians in 1999 with detailed field exploration in Argentina and a study of pathogens on both species. New Zealand joined the project soon after: reports on the prospects for biocontrol of both grasses were written by Peter McGregor (Landcare Research) in 1999 and these recommended collaboration with Australia.

The potential biological control candidates identified for serrated tussock were found to be either not host specific (*Puccinia nassellae*), not sufficiently pathogenic to Australian populations of the weed (*P. nassellae, Tranzscheliella* spp.) or their biology and life cycle could not be fully determined (*P. nassellae, Tranzscheliella* spp., *Corticiaceae* sp.), precluding further work .

Concurrently, *Puccinia nassellae* ex CNG and two other rust fungi, *Puccinia graminella* Diet. & Holw. and *Uromyces pencanus* Arth. & Holw., were identified as potential biological control candidates for CNG (McLaren, Anderson et al. In press). *Puccinia nassellae* Arth. & Holw. ex CNG was found to be extremely host specific; to a point where the most promising isolate tested to-date only caused pustules on three out of the seven populations of CNG from Australia that were inoculated. Mass rearing and storage of *P. nassellae* have proven problematic and consequently it has not undergone comprehensive host-specificity testing. *Puccinia graminella* was found to be damaging to CNG and was common and widespread during the initial surveys. However, it later became scarce, probably due to prevailing drought conditions. A large number of CNG plants within each population tested (from both Australia and Argentina) were found to be resistant to this rust, thus complicating mass culturing for host-specificity testing. Given these difficulties, the most promising candidate agent for CNG is now considered to be *U. pencanus*: it was host-specific during preliminary testing; it is easy to mass culture; its spores can be frozen for later use; and, it can be very damaging to CNG populations in the field (McLaren, Anderson et al. In press).

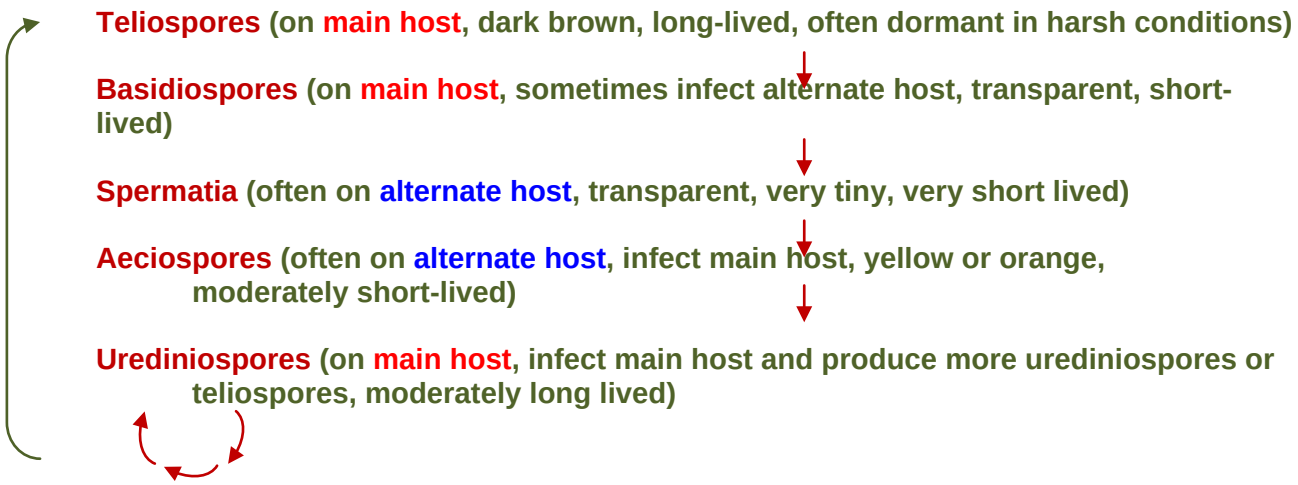
2.2 The use of rusts as classical biocontrol agents: history and safety record

Most of the fungi that have been used for classical biocontrol of weeds are rusts (Basidiomycota: Uredinales), which are obligate parasites (Barton (née Fröhlich) 2004). The characteristics that make rusts particularly useful as classical biocontrol agents are their high virulence, efficient short and long-distance dispersal (via dry, air-borne spores) and their high host-specificity. To-date, none of the rusts (or other fungi) released as classical biocontrol agents for weeds have caused unpredicted damage to a non-target plant (Barton (née Fröhlich) 2004)(JB unpublished talk including data from 2004 to Oct. 2010).

2.3 Life Cycle of Typical Rust

Rusts have very complicated life-cycles that can include up to five spore states (Kirk, Cannon et al. 2008): spermatia (produced in spermogonia), aeciospores (in aecia), urediniospores (in uredinia), teliospores (in telia) and basidiospores (on basidia) (see Fig. 1). A rust fungus may be autoecious, with its life cycle on only one host, or heteroecious, with spermatia and aecia on one host and uredinia and telia on another. Telia produce teliospores which in turn germinate to produce basidiospores (Fig. 1). If basidiospores and the telia they came from are able to infect the same host, then the rust is unequivocally autoecious. If instead basidiospores need an alternate host to complete the cycle, then the rust is heteroecious. Heteroecious rusts are normally not considered as potential biocontrol agents because host specificity testing would have to include species related to each of the two alternate hosts (Morin, Evans et al. 2006), and that would make the process overly laborious.

Fig. 1. Spore Types Potentially Formed by a Rust with a Full Life Cycle.



2.4 U. *pencanus* in Argentina: Field observations

Field surveys to locate and collect isolates of *Uromyces pencanus* from CNG populations were initiated in spring 2003, and continued for five years. Note that other rust fungi encountered on CNG that were thought to have potential as biocontrol agents were also collected during these surveys (Anderson, Barton et al. 2010). A significant part of the geographical distribution of the grass in Argentina has been explored. All populations of CNG encountered while travelling were inspected. Whenever a rust infected plant population was found, infected material was collected in paper bags and dried in a plant press for further study in the laboratory, and a GPS reading was taken to record the site location. Dicotyledonous plant species that grew in close association with rust infected CNG plants were searched for aecia in case one should prove to be an alternate host of arust with biocontrol potential.

Sites were numbered in the order in which they were recorded. Rust isolates were labelled with the initials of their Latin name and the number of the site at which they were collected (e.g. UP 27 was the isolate of *U. pencanus* collected at site 27). Material collected in the field was examined microscopically to identify fungal species and spore types present.

Uromyces pencanus was found infecting CNG plants at 17 sites from Bahía Blanca, Buenos Aires Province in the south, to Alta Gracia, Córdoba Province, in the north. Spores belonging to seven different isolates, including UP27, were collected and stored. Heavy infections of *U. pencanus* alone were observed killing infected foliage, especially under hot, dry conditions. Seed production was often observed to be greatly reduced in rust infected plants as compared with healthy plants at the same site. This was evident both at sites where *U. pencanus* was the only rust observed, and at sites where mixed infections of *U. pencanus* and *P. graminella* were found.

Uromyces pencanus was found to be particularly common at site 27, and since this site is in Bahía Blanca (where the project is based), it was visited frequently (every few weeks) during 12 months in an attempt to follow the life cycle of this rust in the field throughout the year. This site was also visited (more sporadically) over a further four years. At site 27 only uredinia were present most of the time, with peaks of infection in the autumn and spring. Telia developed at the end of spring/beginning of summer.

Both urediniospores and teliospores of *U. pencanus* were found to be common in the field, but aeciospores of that rust were never encountered: not on CNG plants; nor on other plant species growing in association with them.

2.5 Damage to *Nassella neesiana* in Argentina

Pustules of *U. pencanus* urediniospores erupt through and break the epidermis of CNG leaves (Shaw 1963). This would cause increased water-loss through increased transpiration rates (Duniway and Durbin 1971). It is hypothesised that this is why the rust is particularly damaging under dry conditions. An experiment was carried out under controlled conditions in the laboratory with isolate UP 27. 100 % of CNG plants grown from seed collected at site 27 and 70 % of Australian plants (ex ACT) were infected by this isolate (McLaren, Anderson et al. In press). Inoculated plants from Argentina grew significantly less than uninoculated plants of the same accession and Australian plants produced fewer leaves and had a lower dry weight of living material than did uninoculated plants (McLaren, Anderson et al. In press).

3. Biology and ecology of *U. pencanus*

3.1 Taxonomy of *Uromyces pencanus*

The genus *Uromyces* is in the family Pucciniaceae, order Pucciniales (= Uredinales = the rusts), class Pucciniomycetes, Subphylum Pucciniomycotina, Phylum Basidiomycota (= Basidiomycetes) (Kirk, Cannon et al. 2008). The genus contains about 800 species that are parasitic on angiosperms (flowering plants) (Kirk, Cannon et al. 2008).

3.2 Description of *Uromyces pencanus*

Uromyces pencanus was first described by Arthur (1925).

Description below from (Cummins 1971; McLaren and Anderson 2007)

- Pustules of spores form on adaxial side of leaves. Spermogonia unknown
- Aecia reported on a *Nassella* species in the literature but this is now believed to be a misidentification (see section 3.3 below)
- Uredinia cinnamon-brown (darker than those of *Puccinia nassellae*). Uredinia do not contain paraphyses (unlike those of *P. nassellae*)
- Urediniospores (23–)26–30(–36) μm long, (21–)23–27(–30) μm wide, walls (2–)2.5–3.5(–4) μm thick. Spherical, elliptical, broadly elliptical or ovoid (See Fig. 2), echinulate and with (5–)7–8 (–9) scattered pores.
- Telia erumpent, pulvinate, blackish brown
- Teliospores are one-celled, (27–)30–34(–41) μm long, (20–)21–25(–31) μm wide, cell walls much thicker at the apex (5-10 μm) than at the sides (1-2.5 μm), pedicels 30-62.5 μm long.

Fig. 2. Urediniospores of *Uromyces pencanus* A) In pustules on CNG in the field in Argentina B) Under the microscope.

3.3 Life cycle of *Uromyces pencanus*: One host or two?

In the literature *Uromyces pencanus* is said to form aecia, uredinia and telia on the same host, and to be autoecious (Arthur 1925; Greene and Cummins 1958; Lindquist 1982). During field studies, both uredinia and telia of *U. pencanus* were frequently found on CNG, however, aecia and aeciospores that unequivocally belonged to *U. pencanus* were never observed. Interestingly, aecia and aeciospores belonging to another rust, *Puccinia graminella*, were often observed on CNG during field studies. Both fungi were frequently found at the same site, and sometimes, on the same leaf. Therefore, it was hypothesised that the aecia and aeciospores recorded in the literature as belonging to *U. pencanus* in fact belong to the life cycle of *P. graminella*.

Two different methods were used to test this hypothesis: 1) Aeciospores collected in the field from leaves with and without urediniospores of *U. pencanus* were compared morphologically; and, 2) The author who originally reported that all three spore types occurred on the same host deposited plant material in a herbarium to back-up that claim. This material was borrowed and checked to see if they truly contained only *U. pencanus* or if it was possible that they contained a mixed infection of *U. pencanus* and *P. graminella*.

In addition, experiments were performed in the laboratory to try to coax *U. pencanus* to produce all five potential spore stages, either on one host, or on two. Since the rust had been reported in the literature to be autoecious, no work had ever been done previously to try to find an alternate host. The two hosts of heteroecious fungi tend to be quite different taxonomically (e.g. one may be a monocotyledonous grass and the other a dicotyledonous shrub) but the two plants generally live in close association (Kirk, Cannon et al. 2008) Indeed, if a rust requires two hosts to complete its life cycle, it is logical to expect those two plants to be found consistently growing together within the geographic range of the rust. For that reason, dicotyledonous plant species that grew in close association with rust infected CNG plants were searched for aecia in the field.

Comparison of aeciospores found associated with urediniospores of U. pencanus, with aeciospores of P. graminella)

Aecia were harvested from two naturally-infected leaves collected in the field. The leaf collected at site 153 also contained urediniospores of *U. pencanus*. The other, collected at site 64 (at which *U. pencanus* was not present) bore only aecia of *P. graminella*. In both cases aecia were broken open with a needle and their contents were dispersed in a drop of water on a microscope slide for examination. Spore diameter and wall thickness were measured for 30 spores for each sample (Table 1).

The aeciospores from the two collections were found to be morphologically indistinguishable and both sets of measurements were consistent with the description of aeciospore of *P. graminella* in the literature (Table 1).

Table 1. Comparison of aeciospore measurements

	Aeciospores of *P. graminella* at site 64	Aeciospores associated with urediniospores of *U. pencanus* at site 153	Aeciospores of *P. graminella* according to (Cummins 1971)
Spore length	20–26.25(–30) µm	20–25 µm	(18–)22–25(–33) µm
Spore width	17.5–25 µm	18.75–25 µm	(16–)20–23(–28) µm
Wall thickness	2.5–5 µm	2.5–5 µm	(2–)3–4.5(–6) µm

Inspection of herbarium specimens reported to bear aecia of U. pencanus

To our knowledge, the only known samples of aecia belonging to the life cycle of *U. pencanus* were collected in Chile on *Stipa setigera* Presl. (Arthur 1925) [this name has since been synonymised with *Nassella mucronata* (H.B. & K.) Pohl. (Barkworth and Torres 2001)]. These are deposited in the United States National Fungus Collections (BPI) as specimens US0012260, US001262 and US0012263. All three specimens were borrowed and examined microscopically for this study. Results were as follows:

Specimen US0012260: Aecia, uredinia and one telium were found to be present. The telium was situated in line with a row of aecia (which suggests both spore types were from the same infection). Aspect and size of the urediniospores were in agreement with descriptions given in the literature for *U. pencanus*. Size and aspect of aeciospores were consistent with those of *P. graminella* found in Argentina on CNG. Teliospores were obviously bicellular (hence belonging to the genus *Puccinia*) and their size, wall width and pedicel length were all in agreement with those of *P. graminella* (Cummins 1971). This proves that both rust species are present in this specimen.

Specimen US0012262: Aecia, uredinia and a few telia were found. Most of the latter were scattered on the leaf blade and had unicellular teliospores typical of *U. pencanus*, but there was also a young telium growing in very close association with an aecium, that bore a few bicellular teliospores that were typical of *P. graminella.* Therefore, both rusts were also present in this specimen.

Specimen US0012263: Only aecia and uredinia were found. Aeciospores were very similar in appearance to those of *P. graminella* on CNG in Argentina but slightly bigger than is typical. The characteristics of the urediniospores were consistent with those of *U. pencanus*.

The identity of the host plant contained in these specimens was reassessed by comparing the seed present in two of them (US0012260 and US0012262) with seed characteristics in a key to *Nassella* species provided by Torres (1997). They keyed out to *Nassella neesiana* (CNG), not *Nassella mucronata* (H.B. & K.) Pohl.

Inducement of teliospore germination

The ability of freshly collected teliospores to germinate was tested by placing small sections of CNG leaves bearing telia in Petri dishes containing water agar (WA) and incubating them in the dark at 16–

18 ºC. Incubation continued until contaminating fungi grew over the telia, making it impossible to observe the formation of basidiospores (usually 5–7 days).

The teliospores incubated in this way did not produce any basidiospores. This suggests that they were in a dormant state when collected. Many treatments (including those described in seven published papers, and the authors' own adaptations of those methods) were then applied to try to break teliospore dormancy, and so promote basidiospore formation (McLaren, Anderson et al. In press). None of these methods were successful: it was not possible to induce teliospores to germinate and/or form basidiospores. One way of determining whether or not *U. pencanus* could infect any of the plants identified in the field as potential alternate hosts would be to inoculate them with basidiospores that belong to the life-cycle of that rust. Since no basidiospores were ever produced, that was not possible.

Inoculation of CNG plants with aeciospores from potential alternate host

Dicotyledonous plants that consistently grew in close association with CNG plants infected with *U. pencanus* were searched for aecia in the field and if aecia were found, they were collected. Where possible, seeds from the plant bearing these aecia were also collected. Aeciospores were collected at site 148 from the following species: *Modiolastrum gilliesii* (Steud.) Krapov., (Malvaceae), *Clematis* sp., (Ranunculaceae) and *Conyza albida* Willd. ex Spreng., (Asteraceae). At site 108, they were collected from *Monteiroa* sp. (Malvaceae) and *Krapovickasia flavescens* (Cav.) Fryxell (Malvaceae). The viability of the aeciospores was assessed by placing a sample on water agar in petri dishes, incubating the plates at 18 ºC and in darkness for 24 h, and then assessing the proportion of aeciospores that had germinated.

Of all the aeciospores collected, only those from *Modiolastrum* proved to be viable, and their germination rate was quite low (1-20%).

Four CNG plants grown from seed collected at site 148 were inoculated with aeciospores from *M. gilliesii* from the same site. Small aggregations of dry spores were transferred onto CNG leaf blades under the stereomicroscope and the inoculated plants were then misted with water. The plants were kept in a humidity chamber (100 % RH) for 48 h and later transferred to a controlled environment cabinet at 18-20 ºC with a 12 h photoperiod and around 70 % RH for 20 days, after which they were searched for signs of infection. No pustules developed.

Conclusions regarding the life-cycle of U. pencanus

At the beginning of this study, the authors expected to find aecia, uredinia and telia of *U. pencanus* on CNG in the field. Instead, we found uredinia and telia of *U. pencanus* and aecia of *Puccinia graminella*. It is proposed that the aecia described as belonging in the life cycle of *U. pencanus* (Arthur 1925) belong in fact to *P. graminella.* This hypothesis is supported by the following evidence: 1) aecia were never found in association with uredinia of *U. pencanus*, unless telia of *P. graminella* were also present on the leaf; 2) mixed infections of the two rusts were observed to be common in the field, including instances where the two rusts shared the same leaf; 3) our measurements have shown that aeciospores from a sample infected with *P. graminella* alone and those from one where uredinia of *U. pencanus* were closely associated, are identical (Table 1). Note, this is consistent with a comment by Lindquist (1982): he says that in the absence of teliospores it is impossible to tell the aecia of both rusts apart;, and 4) there are only three herbarium specimens in existence that are reported to contain aecia belonging to the life cycle of *U. pencanus*: two of these (US0012260 and US0012262) unequivocally contain a mixed infection of *U. pencanus* and *P. graminella* and the third one (US0012263) probably does too. The fact that the host in these specimens is actually CNG, not *Nassella mucronata*, further validates our comparison of this material with CNG leaves collected by us in the field.

While it could not be proved the rust is autoecious, no evidence that it is heteroecious was found either. No consistent association was observed between infected CNG and aecia-bearing dicotyledons in the field. The only viable aeciospores collected from a potential alternate host (*M. gilliesii*) did not infect CNG.

Teliospores did not germinate under any of the conditions tested. In the absence of basidiospores, it has not been possible to complete the life cycle experimentally. This same problem was encountered by Ellison et al. (2006) while investigating the life cycle of the rust *Prospodium tuberculatum* (Speg.) Arthur. These authors failed to obtain germination following Evans (1987) and acknowledged that there is in fact a complex of chemical and physical factors involved in the process of inducing teliospore germination. It is of course impossible to test all possible factors and their combinations so that, although unlikely, it is possible that teliospores are able to germinate under conditions not met in our experiments. However, we propose that in nature *U. pencanus* cycles in the form of urediniospores on CNG and that teliospores have lost their capacity to germinate and therefore have no role in the completion of the life cycle. The rust was occasionally observed to become locally extinct at particular field sites (FA pers. obs.), and this is consistent with the loss of functional teliospores from the life-cycle.

To conclude, the weight of evidence collected suggests that *U. pencanus* is autoecious. However, since it is impossible to prove a negative i.e. that an alternate host does not exist, it is possible that it is heteroecious. If an alternate host exists then obviously, its identity is unknown, so it is impossible to know whether-or-not this species, or its close relatives, occur in New Zealand. The authors believe that this risk is the most significant (indeed, perhaps the only one of significance) in this application. Note that if an alternate host does exist in NZ, it will not be an endemic species, since it will be something that grows in the home range of the rust (South America). Note also that Authorities in Australia have previously approved the release of biocontrol agents despite an incomplete knowledge of their life cycle, as it was concluded the benefits of such a release outweighed the potential risks they posed (Evans 1993; Ellison, Pereira et al. 2006).

3.4 *Uromyces* species in New Zealand

An inventory of the rusts that occur in New Zealand was made in 1998 (McKenzie 1998). At that time there were 234 recorded species of rusts here, and 30 of those were *Uromyces* species. Of these 30 species: 18 were adventive (not native to NZ); 5 were indigenous (occurring naturally in NZ and elsewhere); and, 7 were endemic (occurring naturally only in NZ) (McKenzie 1998). Between them, these 30 *Uromyces* species utilise 77 species of hosts plants, a very small proportion (2.7%) of the approx. 2900 plant species that grow in New Zealand (Webb, Sykes et al. 1988). Of these 77 hosts, 28 (36.4%) are grasses. Interestingly, only one *Uromyces* species in New Zealand has been recorded from two quite different hosts (i.e. a main host and an alternate host). That is *Uromyces dactylidis* G.H. Otth which infects two *Dactylis* spp. (Poaceae) and forms aecia on *Ranunculus repens* L. Note that this rust, and all three of its hosts, are adventive in NZ (McKenzie 1998). The other 29 *Uromyces* spp. in New Zealand all have either a single host (12 spp.); or 2 or more hosts that belong to the same genus (13 spp.); or 2 or more hosts on closely related genera in the same family (4 spp.). These figures demonstrate two things that are relevant to this application: 1) it is not at all unusual (in fact, it is the general rule) for *Uromyces* species to have reduced life cycles that do not include all five spore types and do not include an alternate host and 2) that *Uromyces* species tend to be very host specific.

Two other observations of relevance here are made by McKenzie (1998). Firstly, "none of the 17 exotic rusts infecting native plants (in NZ} is of economic or conservation concern" and "Since 1945, on average, more than one new adventive rust has been found per year (in NZ)". Thus, it is unlikely that the addition of *U. pencanus* to New Zealand, a rust that has been through vigorous host range testing (unlike all the rusts that have arrived to-date), would cause any problems in NZ.

3.5 Mycoparasites

A mycoparasite was found covering the pustules on some of the plants inoculated with *U. pencanus* during the early stages of the mass rearing process. It was identified as *Simplicillium* sp., formerly within *Verticillium* section *Prostrata*, a group known to comprise, among others, species which can attack other fungi (Anderson, Barton et al. 2010). While this mycoparasite was not obvious in the field, it thrived under our experimental conditions, thus interfering with the multiplication of spores.

Storage of spores in the freezer was found to eliminate the mycoparasite from our system, as its conidia do not survive such low temperatures. There is still insufficient information to allow the assessment of the impact of hyperparasitism on both fungal and plant populations in nature, but at least in some cases, it has been shown that naturally occurring hyperparasites can significantly reduce the infection pressure of pathogens on their host plants (Anderson, Barton et al. 2010). Field infection of *U. pencanus* by *Simplicillium* sp. has not been observed in Argentina, but nevertheless care should be taken to ensure that *U. pencanus* isolates to be introduced into Australasia are free from this mycoparasite. Since it has not been possible to identify this organism to the species level, it is not possible to know whether or not it already occurs in New Zealand. In any case, areas infected with CNG in New Zealand are unlikely to provide the very high humidity environment that allowed this particular mycoparasite to thrive in the laboratory in Argentina.

The main mycoparasite know to be in New Zealand is *Eudarluca caricis* (Fr.) O.E. Erikss. (teleomorph or sexual stage name) which is also known as *Darluca filum* or *Sphaerellopsis filum* (anamorph or asexual stage name). This fungus is probably worldwide in distribution. It is very common in New Zealand on both introduced and native rusts (Eric McKenzie, Landcare Research, pers. comm.). This mycoparasite occurs in Argentina and was found on rust pustules on *Nassella* species in the field (FA Pers. obs.). It was found to be quite common on pustules of *Puccinia nassellae*, especially in shady habitat. It was also found on *U. pencanus* pustules, but much less frequently. Importantly, the damage to CNG observed in the field (and reported above) was happening in the presence of this mycoparasite, so there is no reason to believe it will prevent similar levels of attack from occurring in New Zealand.

Another mycoparasite present in NZ is *Tuberculina persicina*, again known on both introduced and native rusts. It is probably not that common, but is found more or less worldwide (Eric McKenzie pers. comm.). The genus *Tuberculina* also occurs in Argentina (FA Pers. obs.).

Various *Cladosporium* species are known to grow on rust pustules, and they commonly do so in New Zealand (Eric McKenzie pers. comm.). They may or may not be specific to rusts. *Cladosporium tenuissimum* Cooke, for example, has been isolated from air in New Zealand. *Cladosporium* species were sometimes found on rust pustules on *Nassella* spp. in the field in Argentina (FA pers. obs.). These were not identified to species level. Once again, it can be said that since these mycoparasites occur in Argentina, and do not prevent *U. pencanus* from damaging CNG there, they should not be a problem in New Zealand either.

It is hypothetically possible that if *U. pencanus* was introduced to New Zealand and a mycoparasite that already occurs here found it to be a particularly susceptible host, and then that mycoparasite could become more common in areas with lots of rust-infected CNG. That might pose a risk of increased mycoparasitism on other rusts in that area. There are two rusts in New Zealand that DOC has listed as Nationally Critical (Eric McKenzie, pers. comm.). One of these, *Puccinia embergeriae* McKenzie & P.R. Johnst. is known only on Chatham Islands sow thistle (itself critical), and the other has been found only once (in Taranaki) on *Freycinetia banksii* A. Cunn. (Eric McKenzie, pers. comm.). At present, there are no populations of CNG anywhere near the Chatham Islands or Taranaki. The weed (and therefore the rust) are unlikely to reach the Chathams. Parts of Taranaki may support CNG in future, but most of the Taranaki region is unsuitable for CNG (see Appendix 3). Therefore, there is little risk of increased mycoparasite numbers reducing the populations of these critical native rusts.

Increased mycoparasite populations could potentially reduce populations of other rusts that do live in areas infested with rust-infected CNG. However, it is hoped that introducing the rust will reduce populations of the weed, and that any such spill-over effect will therefore reduce over time. Given that there are over 230 rust species already living in New Zealand, and that the mycoparasites discussed mostly have broad host ranges, it is very unlikely that the addition of a single new rust species would lead to any significant and lasting adverse impact on New Zealand's rust mycota. This is particularly true given the (at present) limited geographic range of the target weed.

3.6 Hybridization

A hybrid is the viable progeny that results from a cross between organisms belonging to two different, but closely related (genus level or below) taxa (Kirk, Cannon et al. 2008). It is unclear how common this process is in nature with fungi but there is good evidence that it has happened (see, for example, (Newcombe, Stirling et al. 2000)).

One hypothetical risk of introducing *U. pencanus* to New Zealand is that it might hybridize with another *Uromyces* species that already occurs here. Two adverse impacts could result from such a scenario 1) the hybrid rust might back-cross with its parent and thus 'pollute' the genetic integrity of a native rust, or 2) the hybrid rust might have a different host range to its parents and therefore pose a risk to non-target plants.

Hybrids can hypothetically be formed in two ways in fungi 1) by anastomosis of germ-tubes, appresoria, substomatal vesicles or intercellular hyphae on the main host or 2) by cross fertilisation of spermogonia on an alternate (aecial) host (Spiers and Hopcroft 1994). Therefore, *U. pencanus* could only form hybrids in New Zealand if it were to infect leaves of a plant that was already infected by another *Uromyces* species. One of the first steps taken in the biocontrol project was a survey of the fungi that already occur on CNG in New Zealand (Fröhlich and Gianotti 2000). No rust fungi were found on the target weed in that survey, and if any had appeared since, it is likely that farmers with infestations of the weed would have informed the authors of that. Therefore, there are no *Uromyces* species on CNG that could hybridize with *U. pencanus* in New Zealand.

In order for hybridization on an alternate host to occur, and for that to lead to an adverse impact, the following events would need to occur: 1) teliospores of *U. pencanus* (which the authors believe to be non-functional) would have to germinate in New Zealand to produce basidiospores, 2) the rust would need to have an alternate host in South America (which the authors were unable to find), 3) that alternate host would have to grow in New Zealand, and to have a geographic range that overlapped with CNG, 4) basidiospores of *U. pencanus* would have to land on that alternate host in sufficient quantities to cause a significant number of spermogonia to form on infected leaves, 5) those exact same leaves would need to also contain spermogonia of another *Uromyces* species (2.7% of NZ's flora is the host of a *Uromyces* species, and most of those do not host the aecial stage) 6) cross fertilization would need to occur, and to lead to a viable aeciospores (this would require the two parent fungi to be genetically compatible, e.g. to have the same number of chromosomes, and compatible alleles), 6) those aeciospores would need to land on a susceptible plant (hybrid fungi reported in the literature often require a hybrid host (see (Spiers and Hopcroft 1994)), and 7) finally, this would all need to happen so often that population level impacts could occur on either native rusts or non-target plants. Therefore it is highly improbable (though not impossible) for the introduction of *U. pencanus* to cause negative impacts through hybridization.

3.7 Evolution of host range

The "risks of increased nontarget use, host addition, or host switching through evolution" with respect to fungal pathogens used as classical biocontrol agents were reviewed by Barton (2004). It was concluded in that paper that "The fact that pathogens evolve does present risks of increased target use. However, thorough host-range testing should reveal the fundamental host range of each pathogen, and that should make it possible to give accurate predictions of the magnitude of such risks for each specific project" (Barton (née Fröhlich) 2004). In the case of *U. pencanus*, the fundamental host range of the pathogen is very narrow, and the target weed does not have any very close relatives (i.e. other *Nassella* species) that are native or valued in New Zealand. This makes it very unlikely that *U. pencanus* would evolve the capacity to attack a nontarget plant species here over time.

Note that the only reported case of a fungal biocontrol agent altering its host range through time is the observation that the strain of the rust *Puccinia chondrillina* Bubák & P. Syd. that was released in Australia in 1971 against *Chondrilla juncea* L. was initially able to attack all three forms of the weed there. Over time, it became more specialised and it is now only able to attack one of these, the narrow-leaved form (Cullen 1971 cited in (Barton (née Fröhlich) 2004)). This nicely illustrates the fact that the chance that a pathogen's host range will increase are no greater than those that it will narrow. Also, such a change is no more likely for an exotic (deliberately imported) rust than for a native one. Finally, given that the only *Nassella* species that occur in NZ are unwanted weeds, a minor broadening of host range would be viewed by land managers as a positive development.

3.8 Predicted distribution of *U. pencanus* in New Zealand

In Argentina *U. pencanus* does not occur everywhere that CNG occurs. However, it does occur across a wide range of latitudes and longitudes, which suggests that microclimate may be more important in determining its distribution than larger-scale climate patterns. Thus, it is difficult to predict the distribution of the rust in New Zealand and for the purpose of this application, the geographical distribution of the rust (an obligate parasite) is predicted to mirror the predicted distribution of susceptible CNG (its only known host). That said, the urediniospores of the rust are wind borne, and therefore able to travel long distances.

3.9 Predicted impact in New Zealand

In the absence of any reason to believe otherwise, the impact of *U. pencanus* on CNG in New Zealand is expected to be similar to that on the same plant in Argentina.

4. Determination of the Host range of *Uromyces pencanus*

4.1 Taxonomy of *Nassella neesiana* (CNG)

Common names: Chilean needle grass (CNG)
Family: Poaceae
Tribe: Stipeae
Genus & Species: *Nassella neesiana* (Trin. & Rupr.) Barkworth

There are no native *Nassella* species in Australia or New Zealand. In South America there are three sub-species of *Nassella neesiana.* These are *Nassella neesiana* var. *neesiana*, *Nassella neesiana* var. *gracillor* and *Nassella neesiana* var. *longiasrisata* (McLaren and Anderson 2007). The taxon that grows in Australia and New Zealand is *Nassella neesiana* var. *neesiana* (Edgar and Connor 2000; McLaren and Anderson 2007).

4.2 Selection of plants for host range tests

The selection of plants to be included in the host range testing of a classical biocontrol agent usually follows the 'centrifugal phylogenetic' method first proposed by Wapshere (1974), and this project is no exception. Wapshere suggested that decision regarding whether or not to include particular plants should be made on the basis of 1) phylogenetic relatedness to the target weed and 2) the likelihood that they would be attacked by the particular agent being tested (Barton (née Fröhlich) 2004). The first point is based on the theory that the more closely related plant species are, the more similar are aspects of their morphology and chemistry, and the more likely they are to be acceptable hosts to particular pathogens. The second point is really just to ensure if there is good reason to include a species on a test list (e.g. because it has been reported in the literature to be a host of the

potential agent, or it is a particularly desirable species that lives in close association with the target weed) it is not left off purely because it is not a close relative.

Since the most important factor determining which plants would be tested for this project was their phylogenetic relationship to *Nassella neesiana* these relationships (within the Poaceae) are shown in Figure 3. Since the rust was known to be quite host specific, and would be rejected as a potential agent if it attacked more than a small number of very closely related grasses, it was not deemed necessary to test species outside the grass family. The degree of phylogenetic separation is shown in the numbers within the squares of the phylogenetic tree (Figure 3) and these numbers are repeated in column 1 of Table 2.

The Poaceae is a very large family that includes many commercially important species as well as valued natives in both Australia and NZ. Consequently, the list of potential species to be tested was very long with 70 non-target plant taxa (including 7 varieties of wheat). Forty-three of those (61.4%) species grow in both countries, and the remainder, just in Australia. There was no reason to believe any unrelated species would be attacked.

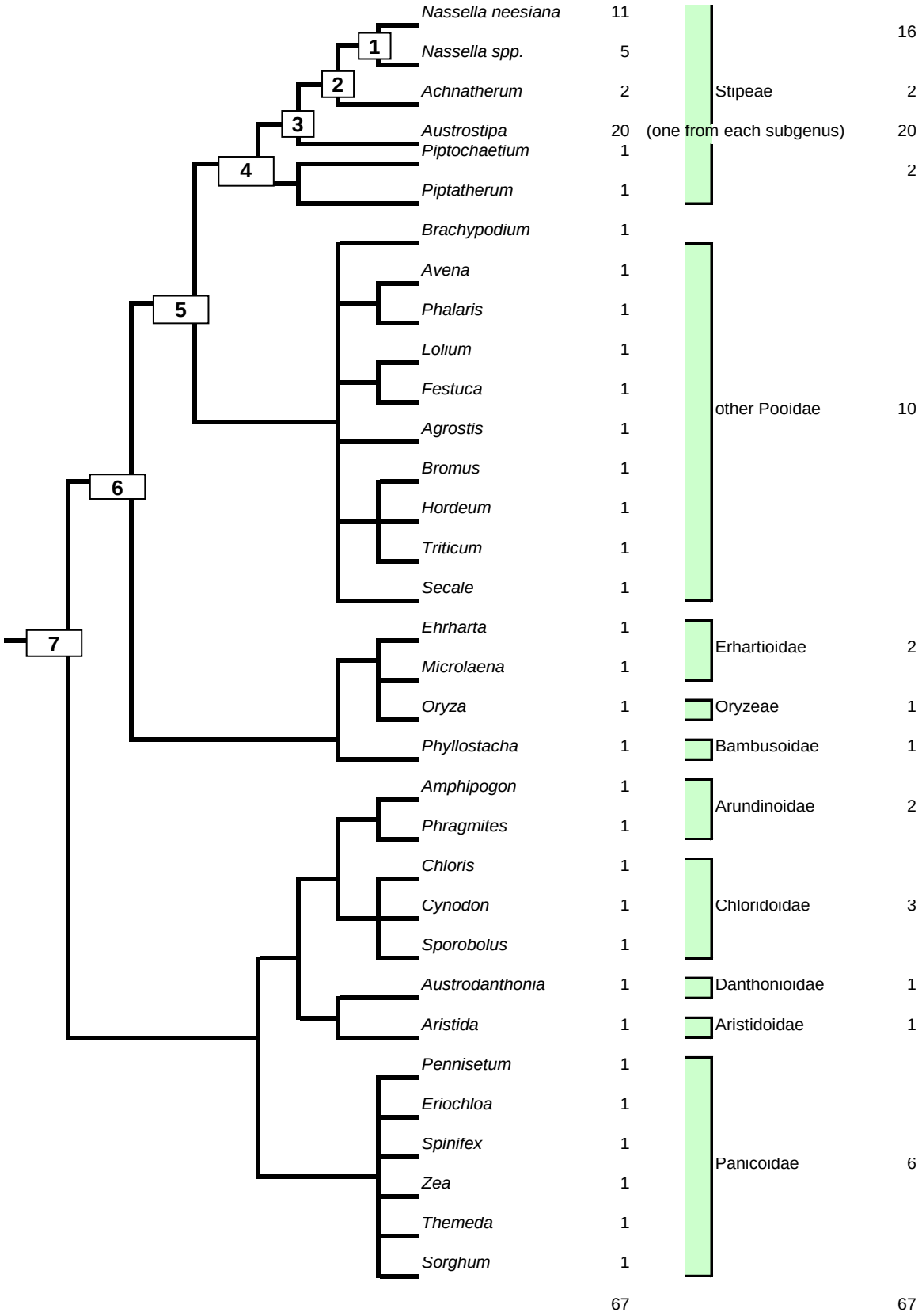


Figure 3. Cladogram showing level of relatedness of host specificity test list genera based on phylogenetic relationships (taken from (McLaren and Anderson 2007))

The closest relatives of *Nassella neesiana* are those grasses that belong in the same tribe, the Stipeae. In NZ there are 5 genera, 18 species and 3 subspecies in the Stipeae. The genera are: *Acnatherum*, *Anemanthele*, *Austrostipa, Nassella* and *Pipthatherum* (Edgar and Connor 2000). Of the 21 taxa within these genera, only three are native (*Achnatherum petriei* (Buchanan) S.W.L. Jacobs & J. Everett, *Anemanthele lessoniana* (Steudel) Veldk. and *Austrostipa stipoides* (Hook. f.) S.W.L. Jacobs & J. Everett) and only the first two of those are endemic (*A. stipoides* also grows in Australia). The remaining 18 taxa are all not only exotic (= adventive) but have also naturalised. Since naturalisation is the first step towards becoming a weed, this gives testament to the invasiveness of this group.

The initial test list of 70 species included representatives of the five genera of grasses from the Stipeae that occur in NZ, including: both spp. of *Achnatherum* that occur here; *Anemanthele lessoniana* (the only species in this monotypic genus); many of the *Austrostipa* species; all three *Nassella* spp. that grow here; and, *Pipthatherum miliaceum* (L.) Coss. (this is exotic and the only member of the genus to grow in NZ). However, due to unforeseen difficulties, some of which are elaborated by Anderson et al. (2010) it proved impossible to test some of these species, and final list of tested plants included the 66 species given below in Table 2. Unfortunately two of the species that it was not possible to test (despite considerable effort) were the two New Zealand endemics *Acnatherum petriei* and *Anemanthele lessoniana.* Fortunately, it was possible to test the other *Acnatherum* species, *A. caudatum* as well as 11 Austrostipa species (including 4 that grow in NZ), all three of our *Nassella* spp. and *P. miliaceum*.

Note that where the level of relatedness was from 1-4 (Figure 3) plants were mostly chosen for inclusion because they are native in Australia and/or New Zealand. Once the plants become quite distantly related within the Poaceae (i.e. levels 5-7) the main criteria for their inclusion became their economic importance. Hence the inclusion of Wheat, Oats, Rye, Barley, and some important pasture species such as Tall Fescue and Perennial Rye Grass.

4.3 Methods for host range tests

Uromyces pencanus isolate UP 27 was selected for host range testing on the basis of its virulence against Australian populations of CNG preliminary tests (Anderson, Díaz et al. 2006). A series of experiments were performed in order to determine optimum conditions for disease development and to develop an inoculation protocol for more comprehensive tests (Anderson, Barton et al. 2010). Batches of 4-5 species were screened at one time, four plants per species with a total of 9-10 plants being tested for each test species (unless otherwise stated in Table 2). Dry urediniospores mixed in talcum powder (ratio 1:30) were brushed onto the adaxial side of leaves, two per plant, which were later sprayed with water. Populations of CNG from the Australian Capital Territory (ACT) were included in each test as positive controls because these proved to be the most susceptible plants available (i.e. they developed more severe disease symptoms than Argentinean CNG plants). Inoculated plants were maintained at 18-20ºC under a 12hr photoperiod and 100% relative humidity (RH) for 48hrs, after which they were kept under the same conditions but at 70% RH for four weeks, double the latent period for infection and sporulation on the positive controls. All inoculated plants were then inspected for external symptoms of infection and samples taken for internal microscopic examination. The samples were stained-cleared using a modification of the Bruzzese and Hasan (1983) method. Each species was screened twice

4.4 Results of host range tests

Table 2. Results of host range Tests

Level of Relatedness	Latin name (origin)	Common name	Status in NZ	Pustules formed?	Growth within leaf cells?	Comments
Target	*N. neesiana* (Hawke's Bay, NZ)	Chilean needle grass (CNG)	Target Weed	No	No	
Target	*N. neesiana* (Auckland, NZ)	CNG	Target Weed	No	No	
Target	*N. neesiana* (Marlborough, NZ)	CNG	Target Weed	Yes	Yes	
Target	*N. neesiana* (ACT)	CNG	Target Weed	Yes	Yes	Most susceptible group. Used as +ve controls
Target	*N. neesiana* (Goulburn, NSW)	CNG	Target Weed	Yes	NE[2]	
Target	*N. neesiana* (Fitzroy flats, NSW)	CNG	Target Weed	Yes	Yes	
Target	*N. neesiana* (Edgars Rd, Vic)	CNG	Target Weed	Yes	NE	
Target	*N. neesiana* (Truganina, Vic)	CNG	Target Weed	Yes	NE	
Target	*N. neesiana* (Ballarat, Vic)	CNG	Target Weed	No	NE	
Target	*N. neesiana (*Bacchus Marsh, Vic)	CNG	Target Weed	Yes	NE	
Target	*N. neesiana* (Laverton, Vic)	CNG	Target Weed	Yes	NE	
Target	*N. neesiana* (Clifton Springs, Qld)	CNG	Target Weed	Yes	Yes	
1	*Nassella trichotoma* (North Canterbury, NZ)	Serrated or Nassella tussock	Weed	No	No	
1	*N. trichotoma* (Dalgety, NSW)	Nassella tussock	Weed	No	No	Yellow leaf spots
1	*Nassella hyaline*	Cane needle grass	Not in NZ	No	No	Yellow leaf spots
1	*Nassella leucotricha*	Texas needle grass	Not in NZ	No	No	Only 2 plants tested
1	*Nassella tenuissima*	Mexican feather grass	Weed	No	No	
2	*Achnatherum caudatum*		Exotic, naturalised	No	No	
3	*Austrostipa aristiglumis*		Not in NZ	No	No	
3	*Austrostipa bigeniculata*		Exotic, naturalised	No	No	
3	*Austrostipa breviglumis*		Not in NZ	No	Yes	Dark leaf spots
3	*Austrostipa elegantissima*		Not in NZ	No	No	Only 5 plants tested Brown leaf spots
3	*Austrostipa eremophila*		Not in NZ	No	Yes	Dark leaf spots
3	*Austrostipa mollis*		Not in NZ	No	No	Only 2 plants tested
3	*Austrostipa nitida*		Exotic, naturalised	No	No	Only 3 plants tested
3	*Austrostipa scabra*		Exotic, naturalised	No	No	
3	*Austrostipa setacea*		Not in NZ	No	No	Only 3 plants tested
3	*Austrostipa tuckeri*		Not in NZ	No	No	
3	*Austrostipa verticillata*		Exotic, naturalised	No	No	Only 2 plants tested
4	*Piptatherum miliaceum*	Rice Millet	Exotic, naturalised	No	Yes	Yellow leaf spots
4	*Piptochaetium napostaense*		Not in NZ (genus not in NZ)	No	No	Yellow leaf spots
5	*Agrostis avenacea* (now = *Lachnagrostis*	NZ Wind grass	Native, not endemic	No	No	

Level of Relatedness	Latin name (origin)	Common name	Status in NZ	Pustules formed?	Growth within leaf cells?	Comments
	filiformis)					
5	*Avena sativa*	Oat	Exotic, naturalised	No	No	
5	*Brachypodium distachyon*	False brome	Exotic	No	No	
5	*Bromus catharticus*		Exotic	No	No	Yellow leaf spots
5	*Elymus scabrifolius*		Not in NZ (genus in NZ)	No	No	Yellow leaf spots
5	*Festuca arundinacea*	Tall fescue	Exotic, naturalised	No	No	
5	*Hordeum vulgare*	Two-rowed barley	Exotic, naturalised	No	No	Yellow leaf spots
5	*Lolium perenne*	Perennial rye-grass	Exotic, naturalised	No	No	
5	*Phalaris aquatica*	Toowoomba canary grass	Exotic, naturalised	No	No	Yellow leaf spots
5	*Poa ligularis*		Not in NZ (genus in NZ)	No	No	
5	*Secale cereale*	Rye	Exotic, naturalised	No	No	
5	*Triticum aestivum* unknown cv.	Wheat	Exotic, naturalised	No	No	Yellow leaf spots
5	*T. aestivum* cv.ACA 303	Wheat	Exotic, naturalised	No	No	
5	*T. aestivum* cv.Arriero	Wheat	Exotic, naturalised	No	No	
5	*T. aestivum* cv.Guapo	Wheat	Exotic, naturalised	No	No	Yellow leaf spots
5	*T. aestivum* cv.Liquén	Wheat	Exotic, naturalised	No	No	Yellow leaf spots
5	*T. aestivum* cv.Malevo	Wheat	Exotic, naturalised	No	No	Yellow leaf spots
5	*T. aestivum* cv.Sureño	Wheat	Exotic, naturalised	No	No	
6	*Oryza sativa*	Rice	Exotic	No	No	
6	*Phyllostachys aurea*	Bamboo	Exotic, naturalised	No	No	
7	*Asistida pallens*		Not in NZ (genus in NZ)	No	No	Yellow leaf spots
7	*Austrodanthonia geniculata*	Kneed wallaby-grass	Not in NZ (genus in NZ)	No	No	Only 2 plants tested
7	*Bothriochloa springfieldii*		Not in NZ (genus in NZ)	No	No	
7	*Chloris gayana*		Exotic, naturalised	No	No	
7	*Cymbopogon citratus*	Lemon grass	Exotic	No	No	
7	*Cynodon dactylon*	Couch	Exotic, naturalised	No	No	
7	*Dichanthium aristatum*		Not in NZ (genus in NZ)	No	No	
7	*Eragrostis curvula*	African lovegrass	Exotic, naturalised	No	No	
7	*Paspalum dilatatum*		Exotic, naturalised	No	No	Yellow leaf spots
7	*Pennisetum clandesinum*	Kikuyu	Exotic, naturalised	No	No	
7	*Phragmites australis*	Common reed	Exotic, naturalised	No	No	
7	*Sorghum halepense*	Johnson grass	Exotic, naturalised	No	No	
7	*Sporobolus rigens*		Not in NZ (genus in NZ)	No	No	
7	*Zea mays*	Sweet corn	Exotic	No	No	

[1] Numbers refer to nodes on Fig. 3. Small numbers = close relatives (i.e. 1 = same genus), larger nos. = less related (7 = same family)
[2] Not examined microscopically

Results of host specificity testing results using *U. pencanus* isolate UP 27 are given in Table 2. The most important result is that pustules of urediniospores only developed on the target weed (CNG). There was some development of the rust within the leaves of three non-target species: *Austrostipa eremophila* (Reader) S.W.L. Jacobs & J. Everett, *A. breviglumis* (J.M. Black) S.W.L. Jacobs & J. Everett and *Piptatherum miliaceum* (L.) Coss. In these plants a few haustoria and some development of intercellular mycelium were observed, however resistance mechanisms (thickening of cell walls upon hyphal contact) were also observed within sections of the same samples. This suggests that the rust cannot complete development and will not persist within these species. Some yellow leaf spots formed on several other species but microscopic studies showed that these resulted from penetration by abnormal hyphae and that hyphal development soon stopped. That is, the plants successfully resisted the infection. Note that environmental conditions for host range testing were optimal for the rust and unnaturally high numbers of spores were applied to the plants tested. Therefore, disease symptoms recorded in Table 2 are a 'worse-case-scenario' with respect to the symptoms that could be expected to occur in the field under more natural environmental conditions.

4.5 Discussion of host range tests

According to the literature, *U. pencanus* has a narrow host range, confined to the genus *Nassella* (Arthur 1925; Cummins 1971; Lindquist 1982). Specifically, it has been reported from: *Nassella chilensis* (Trin.) E. Desv., *Stipa manicata* (now = *Nassella manicata* (E. Desv.) Barkworth), *Stipa mucronata* (now = *N. mucronata* (Kunth.) R.W. Pohl) and *Stipa setigera* (also now = *N. mucronata*) (Barkworth and Torres 2001). Five *Nassella* species were included in the tests reported here, including *N. neesiana* originating from 12 different populations and *N. trichotoma* from two populations. The rust only caused pustules on nine of the *N. neesiana* (CNG) populations. There are about 70 *Nassella* species in Argentina (Barkworth and Torres 2001). Our results are consistent with the literature in suggesting that the rust only damages a very small sub-set of these.

Development of intercellular mycelium and a few haustoria was observed within leaves of *Austrostipa eremophila*, *Austrostipa breviglumis* and *Piptatherum miliaceum* However, the rust did not develop further to form uredinia on these species (Anderson, Gallego et al. 2010). Since it is the formation of uredinia (and the resulting disruption to the leaf epidermis) that is the main cause of damage to the target weed (see section 2.5) these plants would be unlikely to be significantly adversely affected by the rust if they were to encounter it in the field. In any case, those two *Austrostipa* species do not occur in New Zealand and *P. miliaceum* is exotic and not cultivated here.

Austrostipa and *Piptatherum* are both very closely related to *Nassella* (in the same tribe: Stipeae). It is perhaps surprising that no haustoria were observed in *N. trichotoma* or *Achnatherum caudatum* (Trin.) Barkworth which are also in the Stipeae and reported to be genetically closer to CNG (see Fig. 3). Since it was not possible to test the species *Acnatherum petriei*, which is endemic to New Zealand, *Achnatherum caudatum* should be considered a 'surrogate' test species. It is therefore reassuring that *U. pencanus* isolate 27 was unable to penetrate the leaf cells of that species. The genus *Anemanthele* only contains the one species (*A. lessoniana*). Consequently, it was not possible to test another member of the genus as a surrogate for *A. lessoniana*. Instead, we must make inferences from the results from other members of the Stipeae. Since *U. pencanus* was unable to form pustules on any other member of this group (apart from CNG) there is no reason to believe it could do so on *A. lessoniana*

Preliminary testing of five of the seven isolates of *U. pencanus* collected from different sites across Argentina revealed that they varied in their ability to attack different populations of CNG (NB: the last two strains have yet to be tested). Of these five isolates it was decided that UP 27 would be the most useful as a biocontrol agent in Australasia because it was able to infect 6 out of 7 populations of CNG from Australia (more than any of the others). It did

not infect CNG from two populations sent from New Zealand (from Hawke's Bay and Auckland) but fortunately, it did form pustules on CNG plants originating from Marlborough, where New Zealand's worst infestation of the weed occur.

It is possible that further isolates of *U. pencanus* may be wanted in New Zealand in future, to attack CNG in the north island. If so, the question could be asked, "will their host range be consistent with that of isolate UP 27?". The answer to that is "yes" mainly because UP 27 was selected because of the five isolates tested, it had the broadest host range (i.e. attacked CNG from the largest number of populations). Therefore, if isolates of this rust have a spectrum of 'narrow' to 'broad' host ranges within the species *N. neesiana*, UP 27 is at the 'broad' end of it. Also, if an isolate of *U. pencanus* were found that could attack *Nassella* species additional to CNG, results from initial testing of the other isolates suggest it is very unlikely that it would attack members of another genus. It is reiterated that all of the *Nassella* species that occur in New Zealand are unwanted weeds.

Note that in Australia more than one strain of blackberry rust (*Phragmidium violaceum*) has been released. One (F15) was released deliberately in 1991, but one or more strains were also introduced, illegally, in the early 1980s. In this case, the target weed (blackberry) is actually a complex of more than 20 species in the same genus (*Rubus*). Since its introduction the rust has provided useful control of some weedy *Rubus* species in Australia, but not of others. Consequently, a 'trap garden' of resistant blackberry taxa was established in France in 1999 (Morin, Aveyard et al. 2006). This yielded eight 'new' *Phragmidium violaceum* strains thought to have potential for biocontrol in Australia. Initial host range testing done before the release of *P. violaceum* strain F15 involved a 'pool' of 15 different isolates of the rust (Evans, Bruzzese et al. 2002). This was to ensure the host range of the 'species' was covered. The illegally introduced strain/s was/were never tested.

Permission was sought to test the 'new' eight strains on just six cultivars of cultivated blackberry, and three species of *Rubus* native to Australia, that had not been tested previously (Evans, Bruzzese et al. 2002). Permission was granted, so presumably authorities in Australia accepted that the original tests did adequately circumscribe the host range of the rust species (not just the isolates tested). Results of the additional tests were consistent with the original tests: commercial cultivars that were susceptible to the original pool of 15 *P. violaceum* isolates were susceptible to at least some of the eight 'new' ones, while those that had been resistant, were still resistant (Morin, Aveyard et al. 2006). The 'new' *Phragmidium violaceum* strains were released in Australia in April 2004 (Morin, Aveyard et al. 2006). Note that since its original release the rust has only ever been reported on *Rubus* species, and only those that were shown to be susceptible in host range tests (Louise Morin, CSIRO Australia, pers. comm.).

To conclude: *Uromyces pencanus* strain 27 is a highly host specific rust and its urediniospores pose no direct threat to non-target plants in New Zealand. It is unlikely that other stains of *U. pencanus*, should they be wanted in future, would pose any more of a threat than UP 27.

Acknowledgements

The authors wish to acknowledge that much of the work reported was performed by technicians who work in Bahía Blanca with FA i.e. Lucrecia Gallego, Andrea Flemmer, Paula Hansen and Gabriel Roth. The New Zealand component of the research was funded by a consortium of Regional/District Councils concerned about CNG and the Department of Conservation.

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