Manaaki Whenua
Landcare Research

Additional host range testing of the Chilean needle grass rust fungus *Uromyces pencanus* on native Stipeae grasses in New Zealand

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1 Introduction

This report briefly outlines the history of the biological control programme against Chilean needle grass in New Zealand and presents the results of the additional host range testing of the rust fungus *Uromyces pencanus* on three native New Zealand Stipeae grasses.

2 Background

Chilean needle grass (CNG) or *Nassella neesiana* is an erect, tufted perennial grass from South America, which can grow up to one metre high in the absence of grazing. Plants form dense clumps, which exclude pasture species and are less palatable to stock, reducing farm productivity. Chilean needle grass seeds have sharp tips that can bore into the eyelids and pelts of animals, resulting in severe animal welfare issues. Managing farms to avoid CNG damage is a serious economic cost to affected farmers.

Chilean needle grass is highly localised in New Zealand but is spreading and has got worse over the past 12 years since the Environmental Protection Agency (EPA) approved the release of the rust. There are active infestations in Hawkes Bay (600 ha), Marlborough (2,800 ha) and Canterbury (220 ha). CNG has the potential to infest 15 million hectares of New Zealand but less than 1% of this is currently invaded. The potential range includes approximately 1 million hectares of high-producing pasture in Canterbury alone (Bourdôt et al. 2010).

Host range testing was undertaken in Argentina with a single strain of a rust fungus *Uromyces pencanus* (isolate UP 27) which was shown to be highly host specific: only forming pustules and spores on accessions of the target weed, and only on 9 of the 12 CNG populations evaluated (Barton et al. 2010). These results satisfied the EPA that the rust fungus was safe when they approved the release of the rust fungus as a biological control agent for the Chilean needle grass in June 2011.

Difficulties arose regarding the issuance of permits to allow biocontrol agents to be exported from Argentina. Landcare Research could not exercise the EPA approval and it expired. When a new application to the EPA was submitted in 2017, further host range test results were available. The Australian authorities had requested testing of more *Austrostipa* species that are native to Australia, and quite closely related to *Nassella*. In those further experiments, the rust fungus was able to produce spores on two non-target species of *Austrostipa*: *A. compressa* and *A. macalpinei* (Anderson et al. 2017).

While neither of these *Austrostipa* species grow in New Zealand, there are three native Stipeae grasses in NZ that belong in the same tribe as *Nassella*: *Achnatherum petriei* and *Anemanthele lessoniana* and *Austrostipa stipoides*, and the first two are endemic. The Department of Conservation (DOC) requested additional host testing of *An. lessoniana* and *Ach. petriei*, and of New Zealand sourced material of *A. stipoides* if this has not already been tested. While the general host specificity results give a degree of confidence that

these species would not be at risk, they considered that the greater certainty provided by specificity testing is desirable.

In September 2021 permission to export the rust fungus *U. pencanus* strain UP 27 was granted by the Argentinian government. The first attempt to successfully import living spores into containment failed in January 2022. However, a second attempt was successful and in December 2022 the rust culture was hand carried from Argentina into New Zealand. New Zealand now has the only other living culture of the rust fungus on CNG in containment.

The original application and the EPA decision approving release of CNG rust fungus can be found on the EPA database (2011 ERMA approval), while the 2017 application can be found on EPA's website. Subsequently the EPA has approved two-time extensions until April 2024.

3 Objectives

To complete the additional host range testing of three native New Zealand Stipeae grass species *Achnatherum petriei* and *Anemanthele lessoniana* and New Zealand accessions of *Austrostipa stipoides.*

4 Materials and Methods

4.1 Plant and rust fungus propagation

Rust fungi are biotrophs and can only be cultured in living plant tissues. *Uromyces pencanus* inoculum is produced by establishing a susceptible population of *Nassella neesiana* plants in the growth rooms of the PC2+ Beever Plant Pathology Containment Facility (BPPCF).

Nassella neesiana plants, sourced from Marlborough, were grown from seed sourced from plants grown in Manaaki Whenua plant nursery in Lincoln. The spiky awnings were removed by hand and seeds were placed in petri dishes lined with wet filter paper, sealed with parafilm, and left on a glasshouse bench until the hypocotyls emerged. The seedlings were then planted individually in 10-cm-diameter plastic plant pots containing a 1:1 mixture of potting mix and local soil.

Austrostipa stipoides plants were obtained from Kaipara Coast Plant Nursery, Kaukapakapa. The *Ach. petriei* and *An. lessoniana* plants were sourced from the Manaaki Whenua plant nursery in Lincoln.

A culture of the rust fungus *U. pencanus* (isolate UP 27) was imported into New Zealand from Argentina in December 2022. The dry urediniospores, collected in Argentina, were mixed in talcum powder (ratio 1:30) and inoculated onto the leaves of healthy *N. neesiana* plants with a fine paint brush. Inoculated plants were misted with an atomiser and kept in dew chambers [100% relative humidity (RH) at 18–20°C] for the first 48 h. After 48 h the

plants were removed from the dew chambers and kept in the growth room at 18–20°C. When pustules developed, 15–20 days after inoculation, urediniospores were harvested using a mini cyclone spore collector (Anderson et al. 2010; Figure 1).

4.2 Host range methodology

The three test plant species, *Achnatherum petriei*, *Anemanthele lessoniana*, and *Austrostipa stipoides* were screened together at the same time with four plants per species (Table 1). The experiment was repeated three times, totally 12 replicates per test species (n=12). *Nassella neesiana* plants (Marlborough accession) were included in each test as positive controls as the susceptible host.

Dry urediniospores (see Section 4.1), were mixed in talcum powder (ratio 1:50), and were brushed onto the upper leaf surface (10 strokes per leaf) of two leaf blades per plant to a maximum of 20 cm per leaf for *N. neesiana* and *An. lessoniana*. For the *A. stipoides* and *Ach. petriei*, with rolled and smaller leaves, the spore/talc mix was applied to four leaves to add to a total inoculated length of 40 cm per plant. The use of the talcum powder allowed for an even distribution of the inoculum. Whenever possible, intermediate leaves (i.e. not the youngest or the oldest) were selected for inoculation.

The plants were misted with water after inoculation and plants were maintained at 18-20°C and 100% RH for 48 h. They were kept at the same temperature for four weeks, double the latent period for infection and sporulation on the positive controls. All inoculated plants were inspected for external symptoms of infection. The *N. neesiana* control plants consistently developed signs of disease within 15 days post inoculation.

5 Results

Results of the host range testing of *U. pencanus* (UP 27) are presented in Table 1. The most important result is that pustules of urediniospores only developed on the target host, *N. neesiana* (Figure 1a). Chlorosis (yellowing) was observed on a few leaves of *An. lessoniana* (Figure 1b) and chlorotic leaf spots were observed on *A. stipoides* leaves (Figure 1c). The *Ach. petriei* plants did not develop any pustules or signs of disease.

It is important to note that environmental conditions for host range testing were optimal for the rust fungus. Unnaturally high numbers of spores were applied to the plants tested. Disease symptoms and signs recorded in Table 1 are a 'worse-case-scenario' with respect to the symptoms that could be expected to occur in the field under more natural environmental conditions.

Table 1 Results of host range tests

Plant species (origin)	No. or diseased plants/No. of tested plants per repeat	Macroscopic symptoms and signs [
Nassella neesiana (Marlborough)	4/4; 4/4; 4/4	Pustules [100%]
Achnatherum petriei	0/4; 0/4; 0/4	None [0%]

Anemanthele lessoniana	0/4; 0/4; 0/4	No pustules; Chlorotic leaf spots [25%]
Austrostipa stipoides	0/4; 0/4; 0/4	No pustules; Leaf spots [25%]

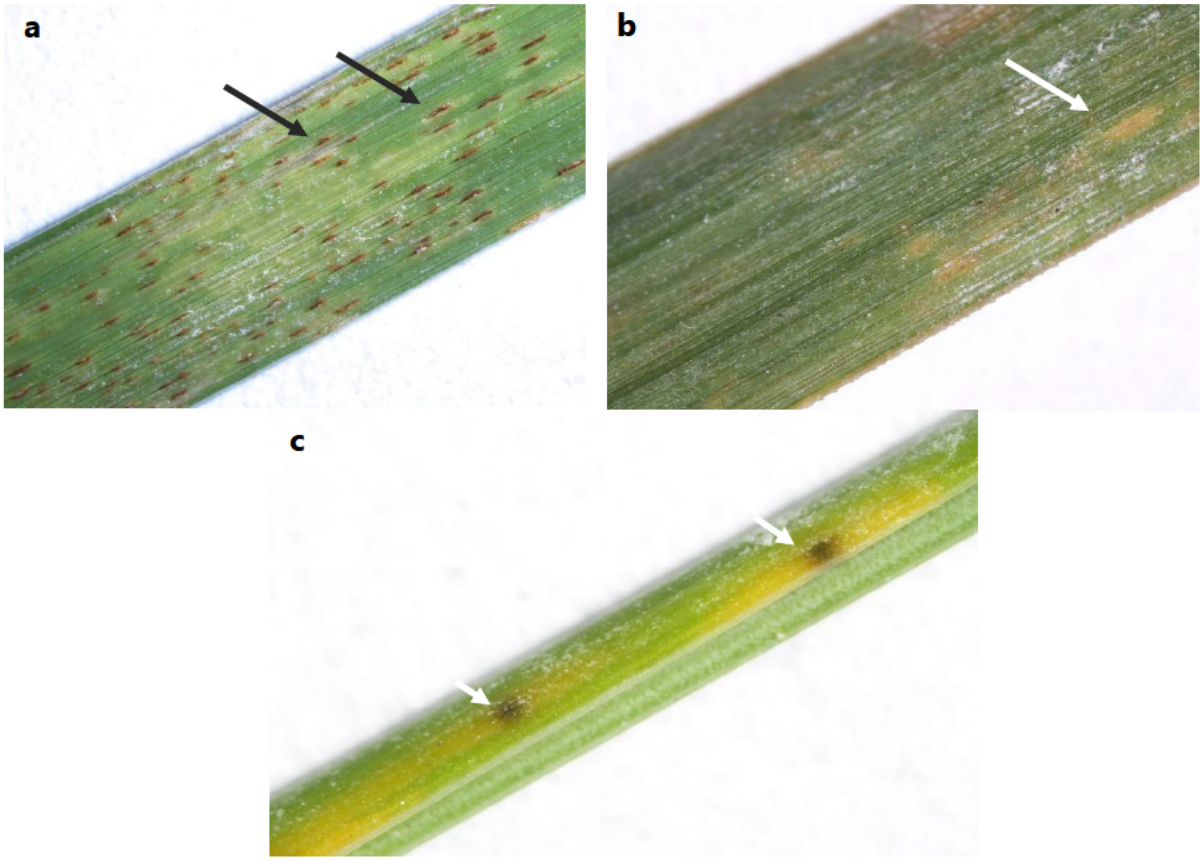

Figure 1. Macroscopic symptoms (black arrows) and signs (white arrows). (a) *Nassella neesiana*: pustules;(b) *Anemanthele lessoniana*: chlorosis; (c) *Austrostipa stipoides*: chlorotic leaf spots.

6 Conclusions

The rust fungus only caused symptoms i.e., pustules on the highly susceptible *N. neesiana* originating from Marlborough. Signs of yellowing and leaf spots were observed on the *Anemanthele lessoniana* and *Austrostipa stipoides*, respectively. No signs of disease were observed on the *Achnatherum petriei*.

Similar signs were observed on *A. stipoides,* and other species as detailed in previous host range studies by Barton et al. 2010 and Anderson et al. 2017.

In Barton et al. (2010), the list of test plants included 11 *Austrostipa* species, including four that grow in NZ, all three of the NZ *Nassella* spp. and *P. miliaceum*. Some development of the rust fungus was observed within the leaves of three non-target species: *Austrostipa eremophila*, *A. breviglumis* and *Piptatherum miliaceum*. However, resistance mechanisms observed within the same samples suggested that the rust fungus cannot complete its development and will therefore not persist within these species. Some yellow leaf spots formed on several other species, but the yellowing was shown to have resulted from penetration by abnormal hyphae and that hyphal development soon stopped. That is, the plants successfully resisted the infection.

In Anderson et al. (2017), the test plant list included three accessions of *N. neesiana* and *Nasella trichotoma* from New Zealand, several *Austrostipa* sp., and an Australian accession of *Austrostipa stipoides*. The rust fungus produced pustules on the Marlborough and Canterbury *N. neesiana* populations only, did not penetrate the *N. trichotoma,* and brown leaf spots were observed on the Australian *A. stipoides* (Figure 2). It was found that in most of the tested taxa, one or more defence mechanisms occurred in response to infection by the rust fungus. The only exceptions were the susceptible accessions of the preferred host *N. neesiana.* These defence mechanisms did not prevent penetration of host tissues, which occurred to some degree in most of the species tested.

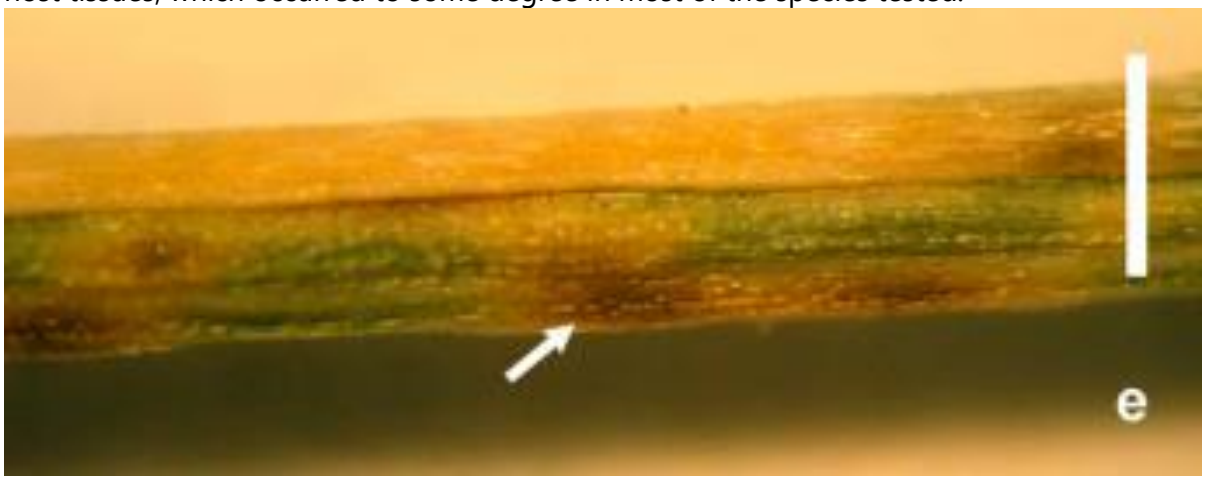

Figure 2. Macroscopic signs (white arrow) of *Austrostipa stipoides*: leaf spots, scale bar: 1 mm (Source: Anderson et al. 2017).

The results of the current host-range testing of the native and endemic NZ Stipeae grasses, *Ach. petriei, An. lessoniana,* and NZ accession of *A. stipoides* provides compelling evidence that the host range of the *U. pencanus* is specific to the Marlborough population of *N. neesiana*.

To conclude: The introduction of *U. pencanus* to New Zealand is highly unlikely to cause any significant negative impact on the native Stipeae grass species or any other native plant or fungus.

7 References

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