Biological Control of Serrated Tussock and Chilean Needle Grass

Pub. No. 11/040
Biological Control of Serrated Tussock and Chilean Needle Grass

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October 2011
RIRDC Publication No 11/040
RIRDC Project No AWRC 08-18
Foreword

_Nassella trichotoma_ (a perennial drought-resistant tussock grass) and _N. neesiana_ (Chilean needle grass, a long-lived tussock-forming grass) are serious agricultural and environmental weeds in Australia and New Zealand and are Australian weeds of national significance.

A biological control program was initiated in Argentina during 1999 and, after several years of detailed field exploration, several potential biological control agents were identified for both _N. trichotoma_ and _N. neesiana_.

To date the most promising agent is _U. pencanus_ since thus far it has been host specific, it is easy to mass rear, its spores can be frozen for later use, and it can be very damaging to _N. neesiana_ populations in the field.

If current host specificity testing continues to be successful, the next phase of this project will involve importation of _U. pencanus_ followed by mass rearing, release and evaluation in Australia and New Zealand.

This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is now publishing the final reports of these projects.

Phase 2 of the Program, which is funded to 30 June 2012 by the Australian Government, is being managed by RIRDC with the goal of reducing the impact of invasive weeds on farm and forestry productivity as well as on biodiversity. RIRDC is commissioning some 50 projects that both extends on the research undertaken in Phase 1 and moves into new areas. These reports will be published in the second half of 2012.

This report is an addition to RIRDC’s diverse range of over 2000 research publications which can be viewed and freely downloaded from our website www.rirdc.gov.au. Information on the Weeds Program is available online at www.rirdc.gov.au/weeds

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Executive Summary

_Nassella trichotoma_ (a perennial drought-resistant tussock grass) and _N. neesiana_ (Chilean needle grass, a long-lived tussock-forming grass) are serious agricultural and environmental weeds in Australia and New Zealand and are Australian weeds of national significance. A biological control program was initiated in Argentina during 1999 and, after several years of detailed field exploration, several potential biological control agents were identified for both _N. trichotoma_ and _N. neesiana_.

The agents for _N. trichotoma_ were, however, not host specific (_Puccinia nassellae_), were not sufficiently virulent for Australian accessions of _N. trichotoma_ (_P. nassellae_ and _Tranzscheliella_ spp.) or their biology and life cycles could not be fully determined (_P. nassellae, Tranzscheliella_ spp. and _Corticiaceae_ sp.), precluding further work.

Three rust species—_Uromyces pencanus, Puccinia nassellae ex N. neesiana_ and _P. graminella_—have been identified as potential biological control candidates for _N. neesiana_. _Puccinia nassellae ex N. neesiana_ has proven extremely host specific, to the point where only three out of seven Australian _N. neesiana_ accessions have been susceptible. Its spores do not store well after freezing, so cultures must either be kept cycling or be re-collected from field sites, which complicates mass rearing and host specificity testing. _P. graminella_ was found to be damaging to _N. neesiana_ and was initially widespread. It appears, however, to be highly dependent on local environmental conditions: recent drought conditions have made this rust scarce over a wide geographical distribution in Argentina. Another concern is that there appear to be large numbers of _N. neesiana_ plants that are resistant to _P. graminella_ infection, complicating mass rearing and host specificity testing.

To date the most promising agent is _U. pencanus_ since thus far it has been host specific, it is easy to mass rear, its spores can be frozen for later use, and it can be very damaging to _N. neesiana_ populations in the field. If current host specificity testing continues to be successful, the next phase of this project will involve importation of _U. pencanus_ followed by mass rearing, release and evaluation in Australia and New Zealand.
Introduction

Serrated tussock

*Nassella trichotoma*, a perennial drought-resistant tussock grass species, is native to the pampas grasslands of Argentina, Uruguay, Chile and Peru (Parodi 1930; Rosengurtt et al. 1970) and has also been reported from Bolivia (Walsh & Entwisle 1994). It has now naturalised in Australia (see Figure 1), New Zealand and South Africa, while small infestations also occur in England and Italy (Campbell 1982; Stace 1997). In Australia, *N. trichotoma* was first recorded at Yass in New South Wales in 1935 (Campbell & Vere 1995); by 1977 it occupied 680 000 hectares (Campbell 1977), and it now occupies more than 870 000 hectares in New South Wales (Osmond et al. 2008). In Victoria, *N. trichotoma* was first recorded as a 4-hectare infestation at Broadmeadows; by 1979 it had spread to occupy about 30 000 hectares (Lane et al. 1980) and by 1998 it occupied more than 130 000 hectares (McLaren et al. 1998). *N. trichotoma* is also found in Tasmania, where it was first recorded in 1956 (Parsons & Cuthbertson 1992), and is currently spread in scattered populations over an area of about 1700 hectares. Overall, in south-eastern Australia *N. trichotoma* is now widespread, covering more than 2 million hectares (Osmond et al. 2008). The species’ potential distribution in Australia has been estimated at 32 million hectares, with substantial areas of New South Wales, Victoria and Tasmania being at risk of invasion (McLaren et al. 1998).

*Figure 1  Current distribution of *Nassella trichotoma* in Australia*

*N. trichotoma* is a C3 grass, characterised by active growth during winter, spring and early summer and a more dormant (less active) period during summer (Osmond et al. 2008). Flowering and seeding occur during spring and early summer, a single *N. trichotoma* plant being capable of producing more than 100 000 seeds. Seed dispersal occurs by wind (up to 20 kilometres), vehicles and machinery, animals, stock feed and produce, soil and waterways (Osmond et al. 2008). (Figure 2 shows the seed.) It is an insidious weed because it can be easily be confused with other pasture grasses, and land managers might not be aware of the extent of the problem until *N. trichotoma* dominates.

*Figure 2  *Nassella trichotoma* seed*
The species is a proclaimed noxious weed in the Australian Capital Territory, New South Wales, Victoria, South Australia and Tasmania. It has been described as having the potential to cause greater reductions in carrying capacity than any other pasture weed in Australia (Parsons & Cuthbertson 1992). It is estimated that in New South Wales alone N. trichotoma costs land managers more than $40 million a year in control and lost production (Jones & Vere 1998). A conservative figure for the annual cost of N. trichotoma control in Victoria is $5 million (Nicholson et al. 1997).

The only registered herbicides for control of N. trichotoma in pastures are flupropanate, glyphosate and 2,2-DPA. N. trichotoma resistance to flupropanate has now been identified at two locations in Victoria and one location in New South Wales (McLaren et al. 2008). Osmond et al. (2008) describe integrated N. trichotoma management using a variety of methods:

- chemical control—use of broad-acre and spot spraying with strategic rotation of herbicide groups
- cultural control—chipping, hoeing, cultivation and mulching
- competition—cropping, pasture rehabilitation, agro-forestry and native revegetation, and strategic cell grazing of stock to promote pasture competition
- seed spread—strategic use of fire and slashing to prevent seeding
- rabbit fencing and shelter belts and windbreaks, along with vehicle and machinery hygiene to prevent N. trichotoma seed spread
- most importantly, monitoring and follow-up control (chipping out) of any regrowing N. trichotoma plants.

In contrast with the vast literature on the impacts of N. trichotoma on agricultural productivity, there is a remarkable dearth of literature on the species’ environmental impacts. Carr et al. (1992) classified N. trichotoma as a very serious environmental weed, while McLaren et al. (1998) provide examples of vegetation formations invaded by N. trichotoma, including dry coastal vegetation, lowland grassland, grassy woodland, sclerophyll forest and woodland, and rocky outcrop vegetation. N. trichotoma is a weed of national significance because of its serious agricultural and environmental impacts (Thorp & Lynch 2000).

The rapid rate of spread of N. trichotoma, its unpalatability for stock and the difficulty of controlling it by chemical and cultural methods prompted an investigation of the possible use of biological control in Australia.

**Chilean needle grass**

*Nassella neesiana*, a long-lived perennial tussock-forming grass, is indigenous to Argentina, Bolivia, Chile, Ecuador, southern Brazil and Uruguay (Rosengurt et al. 1970). In Australia it is both a serious environmental weed (Carr et al. 1992; McLaren et al. 1998) and a problem weed of agriculture (Grech 2007) and has been classed a weed of national significance as a consequence (Thorp & Lynch 2000). It poses a major problem in New Zealand (Bourdot & Hurrell 1992) and has also been recorded in France, Spain, Italy, Portugal, the United Kingdom (Verloove 2005; Stace 1997), South Africa (Gibbs Russell et al. 1985; Wells et al. 1986) and the United States (USDA ARS 2006). In Australia it was first recorded in Victoria in 1934 (McLaren et al. 1998) and is now widespread in Victoria, New South Wales and the Australian Capital Territory; recent outbreaks have also been identified in Queensland, South Australia and Tasmania (see Figure 3) (Snell et al. 2007).
N. neesiana is a declared noxious weed throughout Australia. Once reproductive, it becomes unpalatable, and sheep stocked at rates as high as 300 dry-sheep equivalent will not graze it (Grech 2007). Rotational grazing of N. neesiana is useful as a management strategy only to reduce seed production if the infestation represents an area that can be grazed in the two-week period between seed-head development and panicle emergence (Grech 2007). The species’ potential economic impact on Victoria was assessed by Morfe et al. (2003). They estimated that Victoria would save $82 million over 30 years in control costs and lost production if it could contain N. neesiana infestations at 2002 levels (estimated at 815 hectares). N. neesiana is, however, difficult to control: Gardener and Sindel (1998) say there is ‘overwhelming evidence’ that it is ‘almost impossible to eradicate’ because of the difficulty of killing mature plants, the size and longevity of the soil seed bank, and the production of basal cleistogenes. In particular, N. neesiana threatens the wool industry through contamination of wool, a deterioration in animals’ condition, and physical damage from the sharp-pointed seeds (see Figure 4) penetrating the fleece, skin and eyes of livestock (Slay 2002).

N. neesiana is also a major environmental weed. It has been rated the most significant weed threat to temperate grassland biodiversity in Australia (McLaren et al. 1998; Groves & Whalley 2002) and ‘the worst environmental weed threatening native grasslands’ (Snell et al. 2007). Faithfull et al. (2009) found, however, that major losses of native plant biodiversity in areas occupied by N. neesiana have probably often preceded the weed’s invasion and have been caused by degrading processes such as Themeda triandra dieback, overgrazing and major soil disturbance. Native plant diversity is greatly reduced inside N. neesiana patches and decreases with increasing patch size (Faithfull et al 2009).
The rapid rate of spread of *N. neesiana*, its unpalatability for stock when it is reproductive, its impacts on biodiversity, and the difficulty of controlling it by chemical and cultural methods have made this species an excellent subject for the possible use of biological control in Australia.
Biological control history

Weedy grasses have only recently become targets for biological control (Witt & McConnachie 2004). In the past they were not considered good targets for a number of reasons, among them the great economic and ecological importance of related species, the simple chemical composition and morphology of grasses (which might preclude any great degree of speciation) and grasses’ great adaptability to grazing and harvesting (Palmer et al. 2008). Broad surveys of the host range of insects showed that grass-feeding species are less specific than those on non-grass hosts, and this led to a recommendation that classical biological control using insects should not be used for grass weeds (Berneys 1985). Previous studies by South African scientists (Wells 1976) on Nassella trichotoma in its native South American range found that insects and pathogens were able to suppress the plant, although the insects were found not to be sufficiently specific. It was therefore decided to assess only pathogens as potential biocontrol agents of N. trichotoma and N. neesiana.

CSIRO commissioned a preliminary survey that was funded by a consortium of shires, councils and state agencies and facilitated by the Victorian Department of Primary Industries and the then New South Wales N. trichotoma Working Group. During three brief visits to Argentina, Dr H Evans (of CABI Bioscience) found a number of pathogens on N. trichotoma, including some related to fungi that had been successfully used for biological control of other weed species (Briese & Evans 1998). Dr Evans’ conclusions were that N. trichotoma had a rich fungal flora in its native range and there was cause for optimism about their potential for biological control. In view, however, of the difficulty of studying the pathogens under laboratory conditions outside their native range, he concluded that a long-term in situ study was required. This would provide a full inventory of the pathogens on the weed, determine their pathogenicity and epidemiology in the field, and lead to an understanding of the biology and life cycles of those showing sufficient specificity to warrant further investigation. A project in South America was developed by the then Cooperative Research Consortium for Weed Management Systems, with the research base being in Bahia Blanca, Argentina, during 1999.
Exploration

The evolutionary centre of the stipoid genus *Nassella* is South America, where it has diversified widely. Of the 98 species of *Nassella*, 90 can be found in South America (Torres 1997). As a result, South America was the logical place to begin a search for highly specific pathogens that had evolved with their host grasses, and the chances of finding such organisms were thought to be promising (Briese & Evans 1998). Detailed surveys were carried out between 1999 and 2008 throughout a large part of the range of the two target weeds in Argentina (see Figure 5). *N. trichotoma* is a common pasture component in the Argentine pampas, an area of fertile soils used extensively for cropping in the central-eastern part of the country. In the drier regions to the west and south of the pampas grasslands the distribution of *N. trichotoma* becomes patchy, as it does in the more northerly and damper areas. The distribution of *N. neesiana* in South America is less restricted. As well as in the pampas, it is found between Tucumán Province in northern Argentina and Chilean Patagonia.

![Figure 5](image.png)

**Figure 5** Location of *Nassella trichotoma* and *N. neesiana* fungi from surveys in Argentina, South America

During the surveys *N. trichotoma* was found to be most common in the region of Sierra de la Ventana, an outcrop of low mountains about 100 kilometres north of Bahia Blanca, while large populations of *N. neesiana* were found to occur commonly around the Sierras de Córdoba, in central-western Argentina. On the basis of field observations of their ability to damage the target plants and an apparent host range within *Nassella* spp., five fungal species were selected for further investigation as classical biological control agents—*Puccinia nassellae*, *P. graminella*, *Uromyces penceranus*, *Tranzscheliella* spp. and a Corticiaceae species.

A rust fungus, *P. nassellae* was often found attacking both *N. trichotoma* and *N. neesiana*. It was observed to kill adult *N. trichotoma* plants at one site during the summer of 1999–2000 and was therefore chosen as the candidate most worthy of attention. The smut fungus *Tranzscheliella* spp. was selected because of its ability to greatly reduce seed production in both *N. trichotoma* and *N. neesiana*. A member of the Corticiaceae was included as a third possible candidate agent because of widespread
infections, leading to plant death, by this pathogen on *N. trichotoma* recorded by Evans during his preliminary survey in 1994 (Briese & Evans 1998). The rust fungi *P. graminella* and *Uromyces pencanus* were chosen as potential biocontrol agents for *N. neesiana* because they were observed to be very damaging in the field and the literature suggested that their complete life cycles might occur on *N. neesiana*. 
The candidates

*Nassella trichotoma*

Three pathogens—*Puccinia nassellae*, the smut fungus *Tranzscheliella* spp. and a Corticiaceae species—were chosen as the best candidates for *N. trichotoma*.

*Puccinia nassellae*

The rust *P. nassellae* has been recorded on both *N. trichotoma* and *N. neesiana*. Levels of infection of *P. nassellae* in the field are very dependent on environmental conditions, ranging from scarcely detectable after prolonged dry periods to severe outbreaks that kill plants under favourable wet conditions (see Figure 6). On *N. trichotoma* the most severe levels of infection have been recorded at shaded sites, where plants would probably experience longer dew periods than they would out in the open. On *N. neesiana* levels of infection seem to be less dependent on shade. *N. trichotoma* plants infected with *P. nassellae* show chlorotic bands on their leaves, sometimes with necrotic centres. Pustules on the adaxial side of blades commonly remain unexposed inside the convoluted leaves (see Figure 7). In contrast, on *N. neesiana* pustules are obvious on the wide-open leaf blades (see Figure 8).
After thorough searches carried out for almost three years and covering most of *N. trichotoma*’s geographical range in Argentina, only the uredinial state of *P. nassellae* has been found on that grass. Both uredinia and telia are, however, commonly formed on *N. neesiana*. Field observations did not provide conclusive evidence about how the rust completes its life cycle in nature, but they suggest it would cycle as urediniospores on both hosts.

No experimental work was possible with the isolates collected from *N. trichotoma* because no telia were ever found on this host. In the case of isolates from *N. neesiana*, teliospores were induced to form basidiospores in the laboratory, but these basidiospores failed to infect *N. neesiana* or the two identified potential alternative hosts inoculated with them, so it was not possible to determine the role of basidiospores in the life cycle of the rust in the laboratory. It is possible that *P. nassellae* behaves differently and completes its life cycle in habitats and/or on hosts other than the ones that were the subject of this study, but it would appear that on *N. trichotoma* it has lost its telial stage and on *N. neesiana* telia are not functional (FE Anderson, unpub.).
*P. nassellae* ex *N. trichotoma* has proven difficult to mass rear. Inoculation procedures are time consuming because spores need to be applied directly on the adaxial side of leaves by unrolling the blades under the microscope. Other, more traditional inoculation methods were explored but yielded very poor results.

Evidence suggests that the strains of the rust attacking *N. trichotoma* differ from those attacking *N. neesiana*, since cross-inoculations of *P. nassellae* between the two grass species failed (Anderson et al. 2002). Moreover, it appears that strains infecting *N. neesiana* are much more specific than those infecting *N. trichotoma*. Infection has been achieved for *N. trichotoma* in artificial inoculation trials, regardless of the geographic origin of plants or inocula, whereas some accessions of *N. neesiana*, have become infected only with spores collected from infected plants from the same accession.

In initial host range tests several grasses were inoculated with urediniospores collected from naturally infected *N. trichotoma* plants from different sites in Argentina. Among the plants tested were several species of stipoid grasses native to Argentina (*N. neesiana, Stipa clarazii, S. gynerioides* and *Piptochaetium napostaense*) and the Australian species *Austrostipa scabra*. None of these showed signs of infection by *P. nassellae*. The other Australian species tested, *A. aristiglumis*, was, however, moderately susceptible to two of the tested isolates of *P. nassellae* (Anderson et al. 2002). In contrast, neither of the two Australian species became infected with the tested isolates of *P. nassellae* ex *N. neesiana*; nor did any of the species native to Argentina that were tested (*Nassella trichotoma, N. tenuiissima, Stipa brachyachaeta, S. clarazii* and *Poa ligularis* (Anderson et al. 2004; Anderson et al. 2005). Plants from six Australian accessions of *N. neesiana* were inoculated with three *P. nassellae* isolates ex *N. neesiana*: only *N. neesiana* plants from the Australian Capital Territory, Laverton and Truganina accessions were found to be susceptible to one of these isolates, PN 27.

**Tranzscheliella spp.**

Both *Nassella trichotoma* and *N. neesiana* have been found infected by a smut whose identity awaits confirmation but that probably belongs in *Tranzscheliella hypodytres* (formerly *Ustilago hypodytes* sensu lato (Vanky & McKenzie 2002). The evidence collected to date suggests that two distinct species infect the two hosts. The smut on *N. neesiana* is most probably *Tranzscheliella hypodytres*, whereas the one on *N. trichotoma* would appear to be a new, hitherto undescribed species of *Tranzscheliella* (Marcin Piatek, pers. comm.). Patterns of teliospore germination and ornamentation are two of the main characteristics used in smut taxonomy (Duran 1972, 1973). The teliospores from *N. trichotoma* germinated readily on water agar through the formation of germ tubes, but no sporidia were formed. Fusion of cells belonging to either the same or different germ tubes was often observed. This fusion gives place to the infective dycaryotic hyphae. Germination tests with spores collected from *N. neesiana* suggest these spores germinate either directly by germ tubes or by forming sporidia.

The symptoms of infection by *Tranzscheliella* spp. in the field are different on *N. trichotoma* compared with *N. neesiana*. *N. trichotoma* has its inflorescences completely replaced by smutted heads (see Figure 9), while in *N. neesiana* smut spores cover the upper internodes of culms, occasionally allowing the formation of some seed (see Figure 10). Notwithstanding, artificially infected *N. trichotoma* plants showed symptoms that resembled those described for *N. neesiana*. 
It has been proved that infection occurs at germination, although infection rates are extremely low. Field observations show that the incidence of the disease on both *N. trichotoma* and *N. neesiana* tends to be low. For *N. trichotoma*, although the smut occurred at most sites during the surveys for this study, it was usually present on only a few isolated plants at each site. Two exceptions were found at two sites where large sections of *N. trichotoma* populations showed high levels of infection and varying levels of inflorescence replacement. At these two sites many plants failed to produce inflorescences bearing germinable seed. In the case of *N. neesiana*, high levels of infection and inflorescence replacement were observed at only one site; at this site a large population of neighbouring *N. trichotoma* plants showed no signs of smut infection whatsoever, suggesting that it most probably was not susceptible to the smut isolate infecting *N. neesiana*.

Several species of stipoid grasses were tested for their susceptibility to different isolates of the smut collected from naturally infected *N. trichotoma* plants. The plants tested were Australian and Argentinean accessions of *N. trichotoma*; *N. neesiana*, *N. tenuis*, *N. tenuissima*, *Piptochaetium napostaense*, *Stipa clarazii* and *Sipa gynerioides* from Argentina; and *Austrostipa scabra* from Australia. Of these, only *N. tenuis*, *N. tenuissima* and an Australian accession of *N. trichotoma* proved
susceptible. Positive control plants of *N. trichotoma* included in these tests showed, however, either no or little infection, so these results are by no means conclusive (Anderson et al. 2002; Anderson et al. 2004). Infection rates of *Tranzscheliella* spp. on *N. trichotoma* in large-scale inoculation experiments were very poor (less than 1 per cent), precluding *Tranzscheliella* spp. as a potential biological control agent until more effective inoculation techniques are developed.

**Corticiaceae**

In some patches of *Nassella trichotoma* plants showed severe dieback, leading to desiccation and death of the affected tussocks. When pulled out of the ground, the infected plants showed root and crown necrosis, together with a white mycelial mat at the ground level on dead leaves (see Figure 11). Fine hyphae, about 2 microns thick with clamp connections, were sometimes associated with much thicker (about 5-micron) hyaline *Rhizoctonia*–like hyphae on which no clamp connections could be found. Whether both types of mycelia correspond to the same fungus remains to be clarified. Associated with these mycelia are white-brownish crust-like structures on which basidiospores form. All attempts at isolation of the pathogen using artificial media have failed. Instead, *Fusarium* sp. has been repeatedly isolated from affected plants.

![Figure 11 Corticium spp. infecting Nassella trichotoma](image)

Trials were set up in both the field and the glasshouse to test host specificity (Anderson et al. 2002). None of the tested non-target grass species became infected, but none of the *N. trichotoma* plants included as positive controls became infected either. It was therefore not possible to draw any conclusions about the specificity of this pathogen. *N. tenuis* plants infected by a *Corticium*-like fungus were also found near Bahia Blanca.

**Nassella neesiana**

Several pathogens were identified on *Nassella neesiana* (Anderson et al. 2010), and *Uromyces pencanus* and *Puccinia graminella* were chosen as the best candidates.

**Uromyces pencanus**

Heavy infections with *U. pencanus* were seen to be killing infected foliage, especially under hot, dry conditions during the study’s field surveys (see Figure 12). Seed production was often seen to be
greatly reduced in rust-infected plants as compared with healthy plants at the same site. This was noticed both at sites where only *U. penceranus* was present and when mixed infections with *P. graminella* were recorded (Anderson et al. 2010). Further, an experiment carried out in the laboratory under controlled conditions with the aim of studying the impact of infection on the growth of plants from site 27 in Argentina and from the Australian Capital Territory produced the following results:

- The inoculated plants from Argentina grew less than the controls.
- The inoculated plants from Australia formed fewer leaves than the controls.
- The dry weight of green material belonging to inoculated plants was much lower than that of the controls.
- The dry weight of dry (dead) material was higher for inoculated plants than for controls.

In this experiment all the plants from site 27 and 70 per cent of the Australian plants became infected (Giordano et al. 2009).

![Figure 12 Rust fungus *Uromyces penceranus* telia infecting *Nassella neesiana*](image)

Methods have been developed for maintaining a pure culture of *U. penceranus* in the glasshouse and for storing urediniospores (Anderson et al. 2010). Storage in the freezer at –70°C proved the best way of keeping spores viable in the long term: it has been shown that they keep well for at least 12 months at this temperature. A mycoparasite covered the pustules on some of the inoculated plants during the early stages of the mass rearing process. It was identified as *Simplicillium* sp., formerly within *Verticillium* section *Prostrata* (Zare & Gams 2001), a group known to contain species that can attack other fungi (Gams & Zare 2001). This mycoparasite was not obvious in the field, but it thrived under experimental conditions, interfering with the multiplication of spores. It was found that if spores of *U. penceranus* were stored in the freezer the mycoparasite was eliminated from the system since its conidia do not survive such low temperatures (Anderson et al. 2010).

According to the literature, *U. penceranus* is autoecious (Arthur 1925; Greene & Cummins 1958; Cummins 1971; Lindquist 1982), with aecia, uredinia and telia on its grass host. But this study found no aecia on *N. neesiana* at those sites where *U. penceranus* was the only rust present. The only sites where aecia were observed were sites where another rust, *P. graminella*, was also present. In addition,
teliospores did not produce basidiospores under any of the treatments tested in the laboratory to promote germination. It appears that the rust would cycle in the form of urediniospores on *N. neesiana* and that teliospores have lost their capacity to germinate and therefore have no role in completion of the life cycle (FE Anderson, unpub.). The infection process of urediniospores of *U. pencanus* in *N. neesiana* was investigated and found to be similar to that of most other rusts (Flemmer et al. forthcoming).

![Microscopic section of Austrostipa aristiglumis showing development of penetrative hyphae (PH) coming from the substomatal vesicle of the rust (SV) where it has entered the leaf through the leaf stomata (St)](image)

Note: See the thickening of the cell walls in response to the rust, preventing further development (white arrow).

**Figure 13** Microscopic section of *Austrostipa aristiglumis* showing development of penetrative hyphae (PH) coming from the substomatal vesicle of the rust (SV) where it has entered the leaf through the leaf stomata (St)

*U. pencanus* appears to have a narrow host range, confined to the genus *Nassella* (Arthur 1925; Cummins 1971; Lindquist 1982; Barkworth & Torres 2001). Inoculation of a long list of plant species within the Poaceae is under way to test them for their susceptibility to the *U. pencanus* isolate UP 27. UP 27 was selected on the basis of its pathogenicity towards most Australian accessions of *N. neesiana*. The results obtained thus far (see Table 1) are promising in that no pustules have developed on any test species other than the target species, *N. neesiana*. There has been some development of the rust within the leaves of *Austrostipa eremophila*, *A. breviglumis* and *Piptatherum siliaceum*, where a few haustoria and some development of intercellular mycelium were observed (see Figure 14).
Note: See the thickening of the cell walls in response to the rust, preventing further development (white arrows).

Figure 14 Microscopic section of a *Piptatherum miliaceum* leaf showing development of mycelium (M) coming from the substomatal vesicle of the rust (SV) where it has entered the leaf through the leaf stomata (St)

Resistance mechanisms (thickening of cell walls on hyphal contact) were, however, also observed in sections of the same samples, suggesting that the rust will not persist in these species.
<table>
<thead>
<tr>
<th>Species</th>
<th>Macroscopic symptoms</th>
<th>Microscopic symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nassella neesiana</em> (ACT) #</td>
<td>Pustules</td>
<td>1, 2, 8</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Goulburn, NSW) #</td>
<td>Pustules</td>
<td>n.e.</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Fitzroy Flats, NSW) #</td>
<td>Pustules</td>
<td>1, 2, 8</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Edgars Rd, Vic) #</td>
<td>Pustules</td>
<td>n.e.</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Truganina, Vic) #</td>
<td>Pustules</td>
<td>n.e.</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Ballarat, Vic) #</td>
<td>None</td>
<td>n.e.</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Bacchus Marsh, Vic) #</td>
<td>Pustules</td>
<td>n.e.</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Laverton, Vic) #</td>
<td>Pustules</td>
<td>n.e.</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Clifton Springs, Qld) #</td>
<td>Pustules</td>
<td>1, 2, 8</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Hawkes Bay, NZ) #</td>
<td>None</td>
<td>1, 2, 5</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Auckland, NZ) #</td>
<td>None</td>
<td>1, 2, (3), (5), 6, 7</td>
</tr>
<tr>
<td><em>Nassella neesiana</em> (Marlborough, NZ) #</td>
<td>Pustules</td>
<td>1, 2, 8</td>
</tr>
<tr>
<td><em>Nassella trichotoma</em> (North Canterbury, NZ) #</td>
<td>None</td>
<td>1, 4</td>
</tr>
<tr>
<td><em>Nassella trichotoma</em> (Dalgety, NSW) #</td>
<td>Yellow leaf spots</td>
<td>1, 2, 4</td>
</tr>
<tr>
<td><em>Nassella hyalina</em> #</td>
<td>Yellow leaf spots</td>
<td>1, 2, (3), 4</td>
</tr>
<tr>
<td><em>Nassella tenuissima</em> #</td>
<td>None</td>
<td>1, 2, (3), 4</td>
</tr>
<tr>
<td><em>Achnatherum caudatum</em> #</td>
<td>None</td>
<td>1, 2, (3), 5, (6)</td>
</tr>
<tr>
<td><em>Piptochaetium montevidense</em> #</td>
<td>Yellow leaf spots</td>
<td>1, 2, 3, (6)</td>
</tr>
<tr>
<td><em>Piptatherum milaceum</em> #</td>
<td>Yellow leaf spots</td>
<td>(1), 2, 7, (8)</td>
</tr>
<tr>
<td><em>Austrostipa aristiglumis</em></td>
<td>None</td>
<td>1, 2, 3, 5</td>
</tr>
</tbody>
</table>
| *Austrostipa scabra*           | None                | 1, 2, (3), 5, (6), (?)
| *Austrostipa bigeniculata*     | None                | 1, 2, 3, (5), 7      |
| *Austrostipa breviglumis*      | Dark leaf spots     | 1, 2, 3, 5, 7, (8?)  |
| *Austrostipa eremophila*       | Dark leaf spots     | 1, 2, 3, 5, 7, (8)   |
| *Austrostipa mollis*           | None                | 1, 2, (3), (7)       |
| *Austrostipa verticillate*     | None                | (1), (2), (7)        |
| *Avena sativa* #               | None                | (1), (2), 4          |
| *Phalaris aquatica* #          | Yellow leaf spots   | 1, 2, 5, (6), (7)    |
| *Lolium perenne* #             | None                | 1, 2, 3, 5           |
| *Festuca arundinacea* #        | None                | 1, 2, 3, 5, (7)      |
| *Bromus catharticus* #         | Yellow leaf spots   | 1, 2, 3, 5           |
| *Hordeum vulgare* #            | Yellow leaf spots   | 1, 2, 5              |
| *Triticum aestivum* unknown cv. # | Yellow leaf spots | 1, 2, 3, 4           |
| *T. aestivum* cv. ACA 303 #    | None                | 1, 2, 3, 5, 6, 7     |
| *T. aestivum* cv. Liquén #     | Yellow leaf spots   | 1, 2, 3, 5, 6, 7     |
| *T. aestivum* cv. Arriero #    | None                | 1, 2, 3, 5, 6, 7     |
| *T. aestivum* cv. Sureño #     | None                | 1, 2, 3, 5, 6, 7     |
| *T. aestivum* cv. Malevo #     | Yellow leaf spots   | 1, 2, (3), 5, 6, 7   |
| *T. aestivum* cv. Guapo #      | Yellow leaf spots   | 1, 2, 3, 5, 6, 7     |
| *Secale cereale* #             | None                | 1, 2, 3, 5, 6        |

Table 1  Host specificity testing results for Poaceae species inoculated with *Uromyces pencanus*
<table>
<thead>
<tr>
<th>Species</th>
<th>Macroscopic symptoms</th>
<th>Microscopic symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phyllostachys aurea</em> #</td>
<td>None</td>
<td>1, (2), (3), 4</td>
</tr>
<tr>
<td><em>Phragmites australis</em></td>
<td>None</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td><em>Chloris gayana</em> #</td>
<td>None</td>
<td>1, (2), 3, 4</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em> #</td>
<td>None</td>
<td>1, 2, 4</td>
</tr>
<tr>
<td><em>Sporobolus rigens</em> #</td>
<td>None</td>
<td>1, (2), 4</td>
</tr>
<tr>
<td><em>Aristida pallens</em> #</td>
<td>Yellow leaf spots</td>
<td>1, (2), 4</td>
</tr>
<tr>
<td><em>Pennisetum clandestinum</em> #</td>
<td>None</td>
<td>1, 2, 5, 6, 7</td>
</tr>
<tr>
<td><em>Zea mays</em> #</td>
<td>None</td>
<td>1, 2, 3, 5, 6, 7</td>
</tr>
<tr>
<td><em>Sorghum halepense</em> #</td>
<td>None</td>
<td>1, 2, 5</td>
</tr>
<tr>
<td><em>Brachypodium distachyon</em> #</td>
<td>None</td>
<td>1, 1a, (2), (3), 7</td>
</tr>
<tr>
<td><em>Oryza sativa</em> #</td>
<td>None</td>
<td>1, 2, 3, 5, (6)</td>
</tr>
<tr>
<td><em>Eragrostis curvula</em> #</td>
<td>None</td>
<td>1, (2), (3), 4</td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em> #</td>
<td>None</td>
<td>(1)</td>
</tr>
<tr>
<td><em>Poa ligularis</em> #</td>
<td>None</td>
<td>1, 2, 3, (5)</td>
</tr>
<tr>
<td><em>Elymus scabrifolius</em> #</td>
<td>Yellow leaf spots</td>
<td>1, (2), 3, (6), (7)</td>
</tr>
<tr>
<td><em>Bothriochloa springfieldii</em></td>
<td>None</td>
<td>(1), (2), (5), (6)</td>
</tr>
<tr>
<td><em>Paspalum dilatatum</em> #</td>
<td>Yellow leaf spots</td>
<td>1, (2), 5, 6, (7)</td>
</tr>
<tr>
<td><em>Dicanthium aristatum</em> #</td>
<td>None</td>
<td>1, (2), 3, 5, 6, 7</td>
</tr>
</tbody>
</table>

Notes: 1—normal spore germination; 1a—abnormal spore germination; 2—normal appresoria; 3—abnormal appresoria, non-stomatal appresoria; 4—penetration not observed; 5—penetration, two to four infection hyphae formed from substomatal vesicle, growth cessation; 6—penetration plus contact with plants cells, growth cessation; 7—penetration plus contact with plant cells plus thickening of cell wall, growth cessation; 8—haustoria, abundant intercellular mycelia; ()—observation infrequent; ?—a doubtful observation; *—only one or two plants of the species tested thus far; #—exotic species to Australia; n.e.—not examined.

**Puccinia graminella**

The rust *Puccinia graminella* was seldom encountered alone during the surveys; it was mostly found in mixed infections with the rusts *Uromyces pencanus* and *Puccinia nassellae*. Notwithstanding, on the few occasions when it was found alone it was seen to seriously damage individual plants by causing premature senescence of foliage. For reasons unknown this rust used to be more common around Bahia Blanca—not only on *Nassella neesiana*, but also on *N. tenuis* and *N. hayliina* (Briese et al. 2000)—but has recently become rare in the region (FE Anderson, unpub.).

Aecia and telia have been recorded on *N. neesiana*, their relative abundance varying with the time of year. It has been shown that aeciospores of this rust are repetitive; that is, infection by aeciospores produces new aecia (unpublished data). Strictly speaking, therefore, these spores should be referred to as aecidioid urediniospores, since the urediniospores are the repeating vegetative spores and the aeciospores are non-repeating by definition (Kirk et al. 2008). It was also found that teliospores of *P. graminella* germinate directly without a dormant period, producing normal-looking basidiospores, which in turn germinate readily on water agar. It was expected that these spores would infect plants of *N. neesiana* to give new aecia, but no infection by basidiospores was achieved under experimental conditions. With the evidence collected to date, the role of the teliospores and basidiospores in the life cycle remains unknown, and it appears the rust cycles as aecidioid urediniospores (FE Anderson, unpub.).

According to the literature, *P. graminella* infects *Nassella* spp., *Stipa* spp. and *Piptochaetium* spp. (Cummins 1971; Lindquist 1982; Mujica & Vergara 1945). The occurrence of high numbers of resistant individuals among the plants used in inoculation experiments did not allow the for
establishment of a pure culture in the laboratory (Anderson et al. 2008). This precluded the performance of significant host specificity experiments. Nevertheless, some preliminary tests were carried out in which *N. neesiana* plants from the Australian Capital Territory were included. A small percentage of these (15 per cent) did become infected.

![Figure 14: Puccinia graminella aecia (yellow) and telia (black) on Nassella neesiana](image_url)
Discussion

To be an effective biological control agent, an organism must have two important characteristics: it must be able to damage the target weed sufficiently to cause population-level effects, either alone or in combination with other agents; and it must possess a degree of host specificity that will not pose unacceptable risks to non-target plant species. What then can be said about the candidate pathogens for *Nassella trichotoma* and *N. neesiana* in this context? Given their apparent inability to cross-infect each other’s host plant, the strains of *Puccinia nassellae* on *N. trichotoma* and *N. neesiana* should be considered pseudo-separate species. High levels of infection by *P. nassellae* have been observed on *N. trichotoma* in the field, and the rust was seen to kill individual host plants. Such events were not common, though, and appeared to be highly dependent on favourable environmental conditions—that is, moist, shaded conditions. Although the rust is able to persist during drought periods, there are reservations about whether it can attain densities sufficient to allow it to have widespread impacts on *N. trichotoma* populations in Australia.

Overwintering teliospores have never been located on *N. trichotoma*, making it impossible to confirm that the fungus is autoecious. Laboratory experiments have also provided conflicting evidence: the strain of *P. nassellae ex N. trichotoma* does not appear to infect the congeneric *N. neesiana*, yet was capable of forming spores on one species of *Austrostipa* (an Australian stipoid genus), albeit showing a much lower infection level. A single infection sporae grew on *Austrostipa aristiglumis* and did not regrow when scratched off; pustules on *N. trichotoma* always regrew after being scratched off. Because of this lack of host specificity—together with the technical difficulties associated with its inoculation and infection of *N. trichotoma* in the laboratory—*P. nassellae* has been ruled out as a biological control candidate for *N. trichotoma*.

Although work on the *P. nassellae* strains infecting *N. neesiana* has been less intense, these biotypes appear to be more effective and specific. Moreover, the formation of telia on this host has allowed the study of the biology of the rust to proceed further. Confirmation of the nature of its life cycle remains elusive though. Inoculation trials using basidiospores as inocula have failed to provide conclusive results (FE Anderson, unpub.). Further host specificity tests are needed, but the evidence collected to date suggests that *P. nassellae* strains from *N. neesiana* are specific to their host, although an isolate capable of infecting most, if not all, of the Australian accessions of the weed is yet to be identified.

The smut *Tranzscheliella* spp. is very effective in preventing infected plants from forming viable seed, and at one locality infection rates were observed to be high enough to reduce seed production of the *N. trichotoma* population to virtually zero. Survey data again suggest, however, that the incidence of disease is usually low in the field in Argentina. Preliminary studies of host specificity suggest that *Tranzscheliella* spp. *ex N. trichotoma* can attack other *Nassella* species. Specificity at the genus level would not limit its potential for biological control in Australia since there are no native or economically useful plants of this genus in the country. Despite this, the taxonomic status of this smut would need to be resolved, and inoculation methods would have to be refined if a decision was eventually made to do further work on this pathogen.

The corticiaceous basidiomycete is the least studied of the three pathogens because of the difficulty of isolating it on artificial media. This has prevented the determination of Koch’s postulates to clarify whether it is in fact a causative agent of the observed field symptoms of tussock decline. Laboratory work points to the possible involvement of more than one pathogen in the patchy decline of tussocks (including *Fusarium* spp.). In addition, no infection has been achieved on trap plants in either the field or the glasshouse, suggesting that conditions leading to infection are not so easily met or that infection is a very slow process. The identity of the pathogen is not yet known; even its position in the Corticiaceae is tentative. Isolation of this fungus is therefore an essential task in order to permit the work needed if we are to answer questions about the fungus’s potential impact and specificity.
*Uromyces pencanus* appears to be an excellent candidate for the control of *N. neesiana*: it has been found causing severe damage to *N. neesiana* in the field; its spores can be frozen and stored for a long time without significant losses in viability; and to date it has been very host specific.

*Puccinia graminella* was considered a good candidate to complement the control by *U. pencanus*. Experimentation had to be interrupted, however, because cultures were lost in the laboratory and recent drought conditions have resulted in the rust not being found again in the field around Bahia Blanca.

In conclusion, the study has led to the identification of several potential biological control agents for both *N. trichotoma* and *N. neesiana*. Problems with virulence, host specificity and life cycle factors have, however, precluded further work on agents for *N. trichotoma*. Detailed host specificity testing is currently being done for the *N. neesiana* rust fungus *U. pencanus*, and it is expected that if this continues to show the agent is highly specific an application to import and release this agent into Australia and New Zealand will be made in the near future.
References


Campbell 1982


Grech, C 2007, ‘Grazing management for the long term utilisation and control of Chilean needle grass (Nassella neesiana)’, PhD thesis, School of Rural Science and Agriculture, University of New England, Armidale, NSW.


Publications from this project


Flemmer, AC, Anderson, FE, Hansen, PV & McLaren, DA 2010, ‘Microscopic observations of a compatible host/pathogen interaction between a potential biocontrol agent (Uromyces pencanus) and its target weed (Nassella neesiana)’, Mycoscience, in press.


Biological Control of Serrated Tussock and Chilean Needle Grass

Pub. No. 11/040

by David McLaren and Freda Anderson

Boneseed is among the 20 Weeds of National Significance in Australia. *Nassella trichotoma* (a perennial drought-resistant tussock grass) and *N. neesiana* (Chilean needle grass, a long-lived tussock-forming grass) are serious agricultural and environmental weeds in Australia and New Zealand and are Australian weeds of national significance.

A biological control program was initiated in Argentina during 1999 and, after several years of detailed field exploration, several potential biological control agents were identified for both *N. trichotoma* and *N. neesiana*.

To date the most promising agent is *U. pencanus* since thus far it has been host specific, it is easy to mass rear, its spores can be frozen for later use, and it can be very damaging to *N. neesiana* populations in the field.

If current host specificity testing continues to be successful, the next phase of this project will involve importation of *U. pencanus* followed by mass rearing, release and evaluation in Australia and New Zealand. This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is now publishing the final reports of these projects.

Phase 2 of the Program, which is funded to 30 June 2012 by the Australian Government, is being managed by RIRDC with the goal of reducing the impact of invasive weeds on farm and forestry productivity as well as on biodiversity. RIRDC is commissioning some 50 projects that both extends on the research undertaken in Phase 1 and moves into new areas. These reports will be published in the second half of 2012.

This report is an addition to RIRDC’s diverse range of over 2000 research publications which can be viewed and freely downloaded from our website [www.rirdc.gov.au](http://www.rirdc.gov.au). Information on the Weeds Program is available online at [www.rirdc.gov.au/weeds](http://www.rirdc.gov.au/weeds).

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