Biological control of serrated tussock and Chilean needlegrass

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Abstract
Surveys of pathogens attacking *N. trichotoma* and *N. neesiana* in South America have identified three pathogens showing potential for biological control. These are a rust, *Puccinia nassellae*, a smut, *Ustilago* sp. and a Corticiaceae basidiomycete fungus. Different strains of the *P. nassellae* have been found attacking *N. trichotoma* and *N. neesiana.* Differences in life cycle and host range indicate that these strains should be treated separately. *P. nassellae* attacking *N. trichotoma* has not attacked *N. neesiana* and vice versa. However, preliminary host specificity testing of *P. nassellae* attacking *N. trichotoma* has shown some low level infection of the Australian native grass, *Austrostipa aristoglumis.* Field observations suggest that the rust on *N. trichotoma* may be autoecious, but as no telia spores have been found, it is not possible to prove the nature of the life cycle on the host. Its impact appears highly dependent on environmental conditions and this will reduce its effectiveness as a biological control agent in drier locations. To date, *P. nassellae* attacking *N. neesiana* has been host specific, less dependent on environmental conditions for attack and its entire life cycle occurs on *N. neesiana. Ustilago* sp. can cause significant reductions in seed production but its incidence in South America is relatively low. Host specificity of *Ustilago* sp is still under investigation but it is known that the *Ustilago* sp. that infects *N. trichotoma* also infects other *Nassella* species. A Corticiaceae fungus that attacks the crowns and roots of *N. trichotoma* has been identified at a few isolated locations. Little is known of its life cycle or host specificity at this stage. Concerns over agent host specificity and effectiveness in weed control are raised.

Introduction
Of the ten species of stipoid grasses that have been introduced into Australia, serrated tussock (*Nassella trichotoma* (Nees) Hack. ex Arechav.) and Chilean needlegrass (*Nassella neesiana* (Trin & Rupr.) Barkworth) are by far the most widespread and damaging, in terms of their impact on the grazing industry and native grassland environments (McLaren *et al.* 1998).

Following its introduction into Australia from South America in the early 1900s, serrated tussock has spread to infest over 1 000 000 ha in New South Wales and Victoria, with the potential to infest a much greater area in south-eastern Australia (McLaren *et al.* 1998). Serrated tussock is indigestible to stock and out competes more favourable pasture species in many situations (Campbell and Vere 1995), and Randall and Vere (1998) have estimated its cost to the Australian grazing industry to be $40 million per year. In addition, Carr *et al.* (1992) consider it to be a serious environmental weed, particularly in lowland grasslands and grassy woodlands.

Also introduced from South America in the first half of the 20th century, Chilean needlegrass is not as widespread as serrated tussock. Although there are no published estimates of its area of infestation, it is spreading rapidly and has the potential to invade much of south-eastern Australia (McLaren *et al.* 1998). Unlike serrated tussock, it can provide feed for grazing stock over winter, though it reduces summer stock carrying capacity (Gardiner and Sindel 1998). Chilean needlegrass is mainly of concern to conservationists because of its ability to invade a range of native grassy ecosystems (Hocking 1998). It has been described as potentially the worst environmental weed of native grasslands in south-eastern Australia (McLaren *et al.* 1998). Both serrated tussock and Chilean needlegrass have been declared as Weeds of National Significance to Australia (Thorp and Lynch 2000).

The expense of conventional control measures, based primarily on the herbicide flupropanate, and the difficulty of achieving control, particularly in economically marginal grazing land and native ecosystems, led to a strong push for biological control from community and landholder organizations in the mid 1990s. Originally, there had been a view that biological control would not be feasible, as serrated tussock was considered too closely related to native *Stipa* spp. to allow the introduction of agents (Wapshere 1990). However, recent taxonomic studies on stipoid grass genera largely dispelled these fears. Moreover, a preliminary survey of serrated tussock in Argentina suggested that plant pathogens with biological control potential for *Nassella* spp. grasses were present in the native range (see Briese and Evans 1998). As a consequence, funding for a biological control project was obtained via a consortium of local government and community organizations, with contributions from the Rural Industry Research and Development Corporation and Meat and Livestock Australia. The CRC for Weed Management Systems, through CSIRO Entomology, established a research base in Bahía Blanca, Argentina, and work to explore for, and study, potential pathogen control agents commenced in September 1999 (Briese *et al.* 2000).

In this paper we describe the work that has been carried out in Argentina to date, provide brief descriptions of candidate biological control agents, and discuss what further work is needed to determine whether they should be introduced into Australia for formal quarantine safety testing.

Exploration for pathogens in South America
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). The chances of finding highly-specific pathogens that have evolved in a similar fashion was therefore considered promising (see Briese and Evans 1998). Detailed surveys were carried out between 1999 and 2001 throughout a large part of the range of the two target weeds in Argentina (Figure 1). *N. trichotoma* is a common pasture component in the area of 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 the south of the pampas grasslands the distribution of *N. trichotoma* becomes patchy, as it does in the more northern and damper areas (Figure 1). The distribution of *N. neesiana* in South America is less restricted. As well as the pampas, it is also found between Tucuman province in northern Argentina and Chilean Patagonia.

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 km north of Bahía Blanca, while large populations of *N. neesiana* were found to commonly occur around the Sierras de Córdoba, in central western Argentina (Figure 1). During these surveys a total of 12 pathogens were collected from the two target *Nassella* species (Tables 1a and b). Based on field observations of their

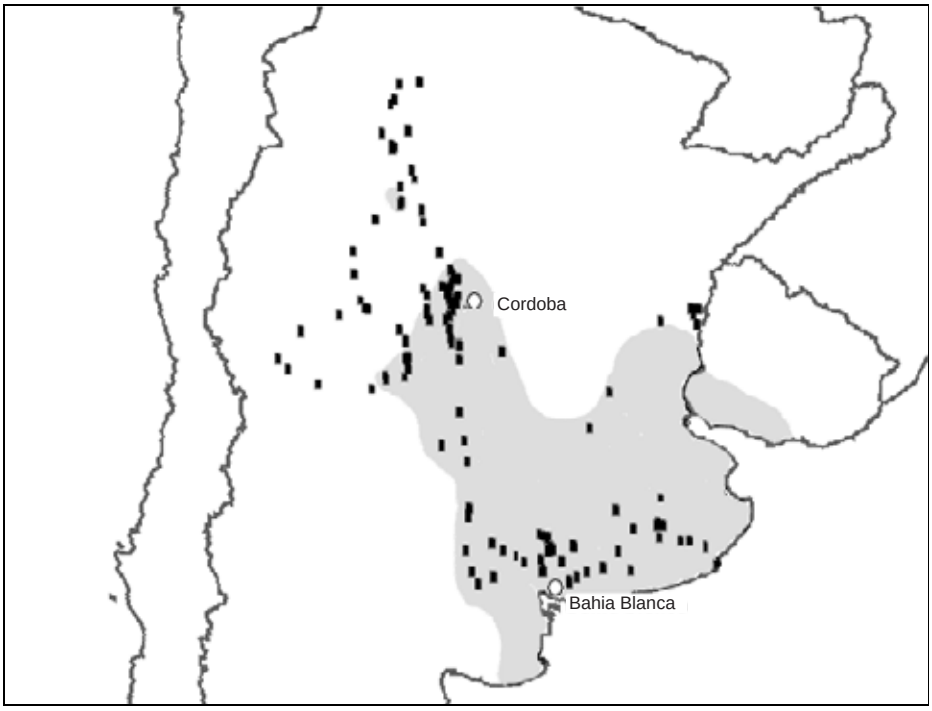

Figure 1. Distribution of *Nassella trichotoma* in South America (in grey) and *Nassella* sites surveyed during 1998–2001 (black dots).

Table 1. Pathogens found on the target *Nassella* species during surveys in Argentina from 1999–2001.

a) *Nassella trichotoma*		
Fungi	**Basidiomycetes**	Corticiaceae
	Uredinales	*Puccinia nassellae*
	Ustilaginales	*Ustilago* sp.
	Coelomycetes	*Fusarium* sp.
		Phoma terrestris
		Zinzipegasa argentinensis
	Ascomycetes	*Blumeria graminis*
		Epichlöe sp.?
	?	Leaf spot
Bacteria		*Clavibacter*? sp. (yellow slime)
b) *Nassella neesiana*		
Fungi	**Basidiomycetes**	
	Uredinales	*Puccinia nassellae*
		P. graminella
		P. aff. *avocensis*
	Ustilaginales	*Ustilago* sp.

ability to damage the target plants and an apparent host range within *Nassella* spp., three fungal species were prioritized for further investigation as classical biological control agents; *Puccinia nassellae, Ustilago* sp. and a Corticiaceae.

Puccinia nassellae, common to both plants and observed to kill adult *N. trichotoma* plants at one site during the summer of 1999 / 2000, was chosen as the candidate most worthy of attention. The smut fungus, *Ustilago* sp., was selected because of its ability to completely abort inflorescences in both *N. trichotoma* and *N. neesiana*, thus preventing seed production. A member of the Corticiaceae was also 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 (see Briese and Evans 1998).

Puccinia nassellae

This rust has been recorded on both target weeds, *N. trichotoma* and *N. neesiana.*

Symptoms Infected *N. trichotoma* plants show chlorotic bands on leaves, sometimes with necrotic centres. Pustules on the adaxial side of blades commonly remain unexposed inside convoluted leaves. It is only when infection is very heavy that the ripening spores exert enough pressure to unroll the leaf and pustules become noticeable (Figure 2). On *N. neesiana*, pustules are obvious on the wide-open leaf blades (Figure 3).

Description Only the uredinial state of the rust has been found on *N. trichotoma*, after thorough searches in the last 2.5 years in the greater part of the plant's geographical range. Another type of spore, morphologically resembling aeciospores of other rusts, has been observed, but, based on available evidence, is currently thought to be an immature urediniospore. Telia are commonly formed on *N. neesiana.*

Life cycle Field observations suggest that the rust is autoecious, completing its entire life cycle on the one host plant species, since no aecia have been observed on other plant species growing in close association with *N. trichotoma*. However, this remains to be experimentally confirmed. As no telia are formed on *N. trichotoma*, it is not possible to prove the nature of the life cycle on this host. Teliospores were collected from *N. neesiana*, as they are readily formed on this host. Unsuccessful germination trials indicated these spores might have a dormant phase, and after trying different treatments, dormancy was finally broken with a cold treatment (*ca.* 4°C for two months), followed by immersion in a 30% hydrogen peroxide solution and rinsing in sterile tap water (Evans 1987). Telia treated in this way were incubated in the darkness at 20°C for several days after which teliospores germinated forming basidiospores (Figure 4). *N. neesiana* plants were inoculated with germinated teliospores but no infection was achieved. These tests will be repeated.

Field impact The level of infection is highly dependent on environmental conditions. On *N. trichotoma*, the most severe levels of infection have been recorded at shaded sites, where plants would likely experience longer dew periods. Serrated tussock leaves are always rolled up with the adaxial face (bearing the stomata) on the inside. *P. nassellae* spores may make their way to the inner surface of the rolled leaf with the aid of water droplets, which would also provide the moisture needed for infection to take place. On *N. neesiana*, levels of infection seem to be less dependent on shade.

Although it was relatively common in the field when surveys first commenced in 1999–2000, the incidence of *P. nassellae* on *N. trichotoma* declined, to the extent where it was difficult to find in the field, during an extended dry period in 2000–2001. However, when overall weather

conditions again appeared to be favourable for rust infection with extended rain periods in spring-summer 2001, the rust reappeared. Table 2 shows the levels of infection observed in the field during surveys in this latter period. *P. nassellae* was present at 74% of the *N. trichotoma* sites (n = 19), but reached levels at which foliage death occurred at only one of these. Infection on *N. neesiana* seemed generally higher, with 86% of sites (n = 14) infected, of which four sites had reached levels at which leaves were killed by the rust.

Host-specificity Evidence indicates that there are different strains of the rust attacking *N. trichotoma* and *N. neesiana*, as cross inoculations of *P. nassellae* between the two grass species failed. 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 for *N. neesiana*, infection only occurred when both plants and spores shared the same origin. More work is needed on the rust infecting *N. neesiana* to better assess its specificity.

Healthy young plants (*ca.* two month-old) of different stipoid grasses were inoculated with *P. nassellae* urediniospores collected from naturally infected *N. trichotoma* plants from different sites in Argentina. Infection of the host *N. trichotoma* occurred in all tests (Table 3, Figure 5). In addition, none of the three South American stipoid grasses from different genera were infected, nor was the Australian species, *Austrostipa scabra* (Table 3). However, under laboratory conditions, the other Australian test species, *Austrostipa aristiglumis*, was moderately susceptible to the strain of *P. nassellae* that naturally infects *N. trichotoma* (Table 3, Figure 6). Two of the four tested strains successfully infected *A. aristiglumis*, though the level of infection was lower than on the original host plant; 11% of young *A. aristiglumis* plants tested and 8% of older plants developed a single normal pustule per plant compared to up to 12 pustules per plant on 62% of *N. trichotoma* plant inoculated (Table 3).

Ustilago sp.

Identification Both *N. trichotoma* and *N. neesiana* have been found infected by a smut whose identity remains to be confirmed, but which would belong in *Ustilago hypodytes sensu lato*. Patterns of teliospore germination and ornamentation are two of the main characters 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, either belonging to the same or different germ tubes, was frequently observed. This fusion gives

Table 2. Observed levels of infection by *P. nassellae* on *N. trichotoma* and *N. neesiana* in Argentina.

Site ID	Site location	*N. trichotoma*	*N. neesiana*
NT 16	Alcira	---	L / +++
NT 30	Villa Ventana	L / +	0
NT 36	Cosquín	L / ++	---
NT 44	Tres Arroyos	L / ++	0
NT 45	Tornquist	H / ++	H / +
NT 52	Coronel Suarez	H / ++	H / ++
NT 62	Tornquist	L / +	0
NT 64	El Crucero	L / +++	L / ++
NT 84	La Cruz	L / +	0
NT 85	Los Reartes	L / +	0
NT 86	La Falda	L / ++	L / +
NT 87	Ongamira	L / +	L / +
NT 88	La Cumbre	L / +	L / +
NT 89	La Cumbre	0	L / +
NT 90	La Cumbre	0	---
NT 91	Villa Fortabat	0	H / +++
NT 92	Tandil	L / +	0
NT 93	Tandil	L / ++	0
NT 94	Napaleofú	---	H / +++
NT 95	Sierra de los Padres	---	0
NT 96	Balcarce	---	0
NT 97	Balcarce	L / +	L / +
NT 98	San Manuel	---	0
NT 99	Tandil	L / ++	H / +++
NT 100	Tedín Uriburu	L / +	0

0 Host absent; H high incidence; L low incidence; --- rust absent; + low levels of damage (rust is only found after thorough searching); ++ medium (rust is obvious); +++ high levels of damage (rust kills foliage).

Table 3. Results of inoculation of a range of stipoid grasses with *P. nassellae*, using urediniospores collected from *N. trichotoma* in Argentina.

Source of inoculum	No. inoculated plants	No. infected plants	No. pustules per plant
NT51	8 *Nassella trichotoma*	7	Not recorded
Orense	7 *Austrostipa aristiglumis*	0	
	7 *Austrostipa scabra*	0	
NT45	10 *N. trichotoma*	6	1–4
Tornquist	10 *A. aristiglumis*	1	1
	10 *A. scabra*	0	
NT45	10 *N. trichotoma*	9	3–12
Tornquist	10 *A. aristiglumis*	3	1
	10 *A. scabra*	0	
NT 45	10 *N. trichotoma*	8	4–11
Tornquist	8 *A. aristiglumis*	0	
	8 *A. aristiglumis* (1 year old)	1	1
	7 *A. scabra*	0	
NT64	8 *N. trichotoma*	3	1
Los Reartes	8 *A. aristiglumis*	1	1
	4 *A. aristiglumis* (1 year old)	0	
	8 *A. scabra*	0	
	8 *Piptochaetium napostaense*	0	
	8 *Stipa clarazii*	0	
NT64	18 *N. trichotoma*	5	1–6
Los Reartes	10 *A. aristiglumis*	1	1
	10 *A. scabra*	0	
	10 *S. gynerioides*	0	
	10 *N. neesiana*	0	
NT52	10 *N. trichotoma*	9	3–17
La Tacuarita	8 *A. aristiglumis*	0	
	7 *A. scabra*	0	
	8 *S. gynerioides*	0	
	8 *N. neesiana*	0	

place to the infective dycaryotic hyphae. Preliminary germination tests with spores collected from *N. neesiana* indicate these germinate either directly by germ tubes or forming sporidia. Teliospores from both species were also examined under SEM to study exospore ornamentation, which proved to be verrucose and very similar in both origins (Figures 7 and 8).

Symptoms The symptoms of infection of *N. trichotoma* and *N. neesiana* by *Ustilago* sp. in the field are different. *N. trichotoma* has its inflorescences completely replaced by smutted heads (Figure 9), while in *N. neesiana*, smut spores cover upper internodes of culms, occasionally allowing the formation of some seed (Figure 10). Notwithstanding, artificially infected *N. trichotoma* plants showed symptoms that resembled the ones described for *N. neesiana*.

Life cycle It has been proved that infection occurs at germination, as was expected.

Field impact Field observations indicate that the incidence of the disease on both *N. trichotoma* and *N. neesiana* tends to be low (Table 4). For *N. trichotoma*, although the occurrence of the smut was common at most sites, it was usually present on only a few isolated plants at each site. Two exceptions were found at sites NT32 and NT55, 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 (NT64). At the same site, a large population of neighbouring *N. trichotoma* plants exhibited no signs of being infected by *Ustilago* sp.

Host-specificity A host-specificity test was performed in which several species of stipoid grasses (*N. trichotoma, N. tenuis, N. tenuissima, N. neesiana* and *Austrostipa scabra*) were inoculated with smut spores collected from naturally infected *N. trichotoma* plants. Unfortunately, the *N. trichotoma* plants used as positive controls did not flower, despite the application of gibberellic acid to encourage flowering. In the test, two *N. tenuissima* plants became infected, showing this species is also susceptible to the strain of smut that naturally infects *N. trichotoma*. This test is currently being repeated under glasshouse conditions.

Corticiaceae

Symptoms Patches of *N. trichotoma* plants showed severe die-back leading to desiccation and death of affected tussocks (Figure 11). When pulled out of the ground, infected plants show root and crown necrosis together with a white mycelial mat at the ground level on dead leaves (Figure 12).

Table 4. Observed levels of infection by *Ustilago* sp. on *N. trichotoma* and *N. neesiana* in Argentina.

Site ID	Site location	*N. trichotoma*	*N. neesiana*
NT16	Alcira	---	L
NT30	Villa Ventana	L	0
NT32	Villa Ventana	H	
NT36	Cosquín	---	---
NT44	Tres Arroyos	---	0
NT45	Tornquist	---	---
NT52	Coronel Suarez	---	---
NT55	Villa Ventana	H	
NT62	Tornquist	L	0
NT64	El Crucero	---	H
NT84	La Cruz	---	0
NT85	Los Reartes	---	L?
NT86	La Falda	---	---
NT87	Ongamira	---	---
NT88	La Cumbre	---	---
NT89	La Cumbre	0	---
NT90	La Cumbre	0	---
NT91	Villa Fortabat	0	L?
NT92	Tandil	---	0
NT93	Tandil	---	0
NT94	Napaleofú	---	---
NT95	Sierra de los Padres	---	0
NT96	Balcarce	---	0
NT97	Balcarce	---	---
NT98	San Manuel	L	0
NT99	Tandil	---	---
NT100	Tedín Uriburu	---	0

0 Host absent; H high incidence; L low incidence; --- smut absent; ? some doubt on identification of host.

Description Fine hyphae, *ca.* 2 µ thick with clamp connections, were sometimes associated with much thicker (*ca.* 5 µ) 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 there are white-brownish crust-like structures on which basidiospores are formed (Figures 13 and 14).

Isolation All attempts of isolation of the pathogen on artificial media have failed so far. Instead, *Fusarium* sp. has been repeatedly isolated from affected plants.

Field impact The fungus has only been found at a few sites affecting only small patches of *N. trichotoma* tussocks. Plants usually die or are very weak within these patches.

Host-specificity Trials were set up both in the field and the glasshouse to test host-specificity. Healthy trap plants belonging to the species indicated in Table 5 were transplanted into the soil where a patch of infected plants had been located (NT06). The site was visited periodically, but no symptoms of infection have yet been recorded on the test plants. In 1999, roots of *Nassella tenuis* infected by Corticium-like fungal mats, were found near Bahía Blanca.

Table 5. Test plants used in field and glasshouse host specificity trials of the Corticiaceae in Argentina.

Test species field	Test species glasshouse
Nassella trichotoma	*Nassella trichotoma*
N. neesiana	*N. tenuis*
N. tenuis	*Austrostipa aristiglumis*
N. tenuissima	*A. scabra*
Stipa gynerioides	*Triticum* sp.
S. speciosa	*Piptochaetium napostaense*
Poa ligularis	
Piptochaetium napostaense	

In the glasshouse, infected tussocks were transplanted from the field into a wooden box, measuring 2 × 1 × 0.5 m, that had debris infected with the Corticiaceae fungus incorporated into the soil. Healthy test plants of the species indicated in Table 5 were also transplanted into the box. No infection has been recorded in the

Figures 2–6. *Puccinia nassellae*.

Figure 2. *N. trichotoma* leaf showing signs of heavy *P. nassellae* infection (Tres Picos).

Figure 5. *N. trichotoma* artificially infected with *P. nassellae* ex *N. trichotoma*.

Figure 3. *N. neesiana* leaf showing signs of heavy *P. nassellae* infection (Bahía Blanca).

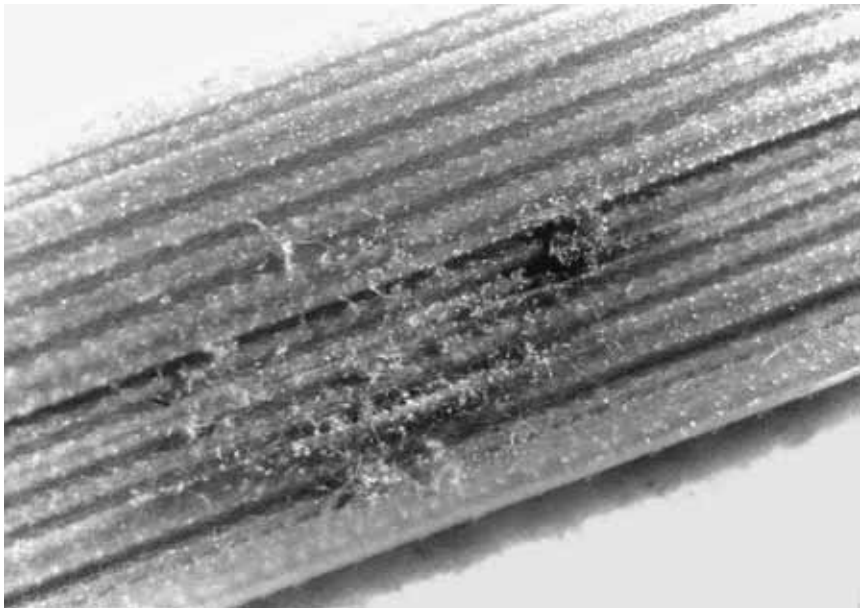

Figure 6. *A. aristiglumis* artificially infected with *P. nassellae* ex *N. trichotoma*.

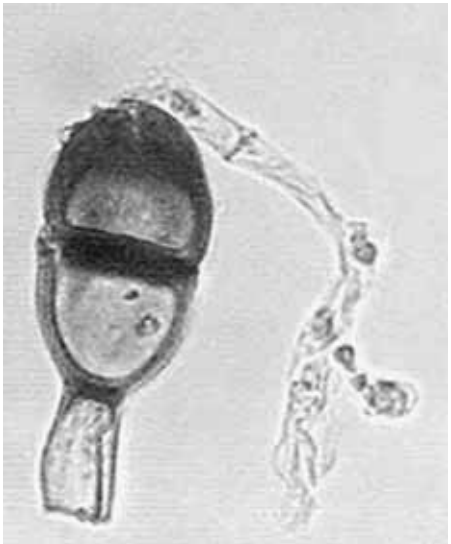

Figure 4. Germinating *P. nassellae* teliospore ex *N. neesiana*.

Figures 7–10. *Ustilago* sp.

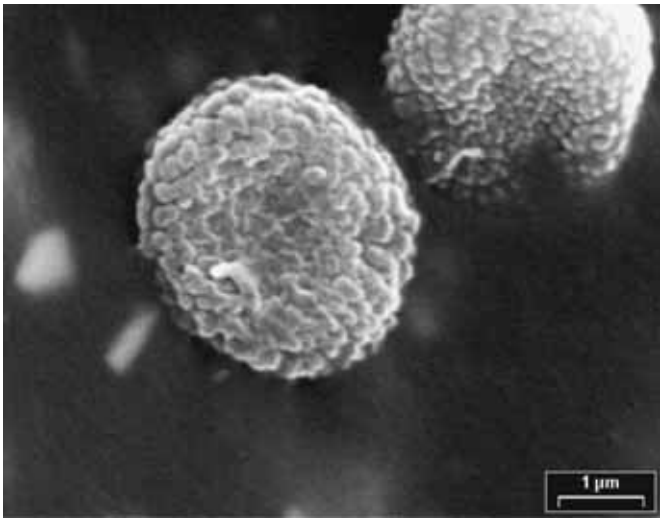

Figure 7. SEM of *Ustilago* sp. spores ex *N. trichotoma* (Villa Ventana).

Figures 11–14. Corticiaceae.

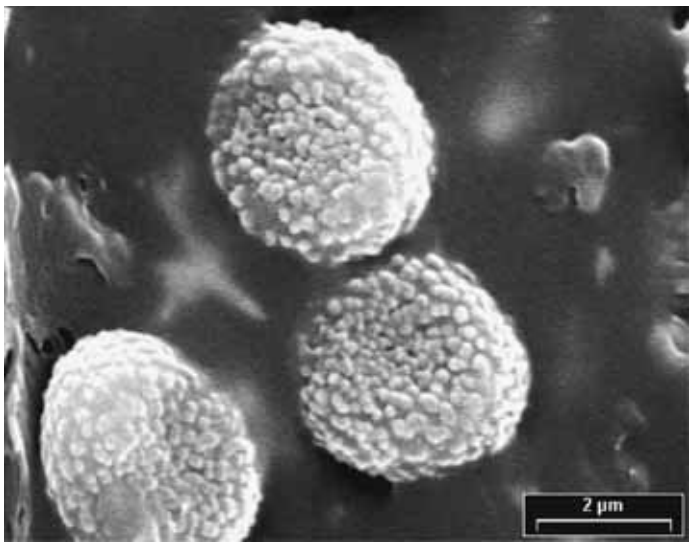

Figure 8. SEM of *Ustilago* sp. spores ex *N. neesiana* (Bahía Blanca).

Figure 13. *N. trichotoma* stems showing the white fungal mat of the Corticiaceae (Villa La Gruta).

Figure 9. *N. trichotoma* inflorescences with seeds replaced by *Ustilago* sp. spores (Villa Ventana).

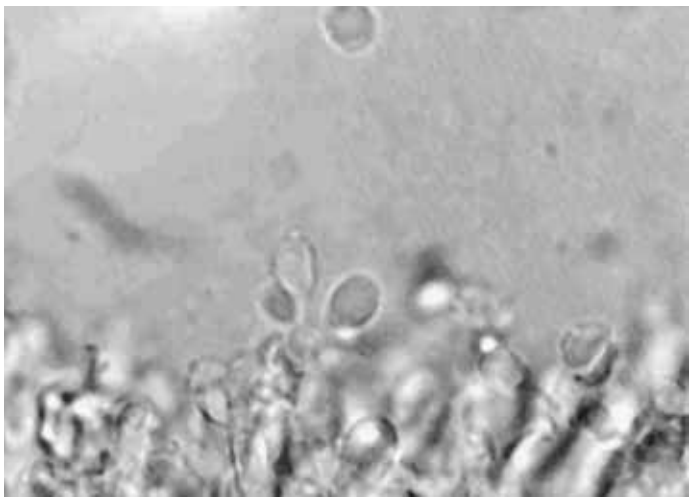

Figure 14. Hymenia of basidia and basidiospores of the Corticiaceae on *N. trichotoma* (Villa La Gruta).

Figure 10. *N. neesiana* inflorescences with reduced seed formation due to *Ustilago* sp infection (Los Reartes).

Figure 11. Adult *N. trichotoma* plant dying back with Corticiaceae infection of the roots (Villa La Gruta).

Figure 12. *N. trichotoma* roots and stems showing the white fungal mat of the Corticiaceae (Villa La Gruta).

following three months to date on any of the species.

Discussion

To be an effective biological control agent, an organism must possess two key 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 risk to non-target plant species. What then can be said about the candidate pathogens for serrated tussock and Chilean needlegrass in this context?

Given the apparent inability to cross-infect each other's host plant, the strains of *Puccinia nassellae* on *N. trichotoma* and *N. neesiana* should be considered separately. High levels of infection by *P. nassellae* have been observed on *N. trichotoma* in the field and it was observed to kill individual host plants. However, such events were not common and appeared to be highly dependent on favourable environmental conditions, i.e. moist, shaded conditions. While the rust is able to persist during drought periods, reservations exist as to whether the rust can attain densities able to have widespread impact on serrated tussock populations in Australia.

Host-specificity of the rust is also problematic, as the overwintering teliospores have never been located, making it impossible to confirm that the fungus is autoecious. Laboratory experiments have also provided conflicting evidence; this strain of *P. nassellae* 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. Clearly, these issues will need to be resolved if it is to be considered for biological control of *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 permits the study of the biology of the rust and the confirmation of its life-cycle. Inoculation trials using basidiospores as inocula are needed to elucidate the nature of the life cycle, while host specificity tests, which include grasses from stipoid genera other than *Nassella*, should demonstrate whether *P. nassellae* strains from *N. neesiana* are specific to their host.

The smut, *Ustilago* sp., is very effective in preventing infected plants from forming viable seed and, at one locality, infection rates were high enough to reduce seed production of the *N. trichotoma* population to virtually zero. However, survey data again indicate that the incidence of disease is usually low in the field in Argentina. Studies on host-specificity have not yet been completed, and a trial of this fungus against seven species of stipoid grasses, including two Australian native *Austrostipa* species, is currently underway in Bahía Blanca, with results anticipated in August 2001 when the plants flower. In a previous glasshouse trial, *Ustilago* sp. ex *N. trichotoma* was found to infect *N. tenuissima*, while casual field observations suggest that *N. tenuis* may also be susceptible. This suggests that *Ustilago* sp. ex *N. trichotoma* can attack a range of *Nassella* spp. Specificity at the genus level would not limit its potential for biological control in Australia, as there are no native or economically useful plants of this genus there. Notwithstanding, the taxonomic status of this smut will need to be resolved, given its close affinity with *Ustilago hypodytes sensu lato*, as *U. hypodytes* is already present in Australia and infects a range of stipoid grasses (Briese and Evans 1998).

The Corticiaceae basidiomycete is the least studied of the three pathogens, due to difficulties with 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 indicates the possible involvement of more than one pathogen in the patchy decline of tussocks (*Fusarium* spp.). Fungi isolated from plant material with Corticiaceae infection formed colonies, which proved to belong to other species, when fruiting bodies were formed on them. In addition, no infection has been achieved on trap plants either in the field or the glasshouse, indicating 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 being tentative. Isolation of this fungus is therefore an essential task to permit the work needed to answer outstanding questions regarding its potential impact and specificity.

In conclusion, the work to date indicates the difficulties that can be expected as we enter a new area of research in seeking biological control solutions for weedy exotic grasses. The project has successfully identified candidate pathogens and provided an important, though incomplete, body of information about their biology, occurrence, and impact in the field. However, in doing so, it has raised some concerns about their usefulness for weed control. Issues concerning their specificity can be resolved through the strict testing protocols that could be undertaken in quarantine in Australia. Sufficient information is available on methodology from the studies in Argentina to test *P. nassellae* and *Ustilago* sp. However, host-specificity testing is an expensive and time-consuming exercise. Any decision to proceed in this direction will need to be based on expert views as to whether the expected impact of the pathogens (alone or in combination) can make this work cost beneficial.

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Variation in size and seed germination in Australian serrated tussock

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Summary

Differences in plant size and seed germination are reported for Victorian *Nassella trichotoma* populations compared to NSW, ACT and Tasmanian populations. Implications for biological control and other control methods are discussed.

Introduction

Nassella trichotoma (Nees) Hack. ex Arechav. is a C_3 perennial tussock-forming grass that is one of the worst weeds that has ever entered Australia's shores. *N. trichotoma* is classified both as an environmental weed and causes a greater reduction in stock carrying capacity than any other weed in Australia (Parsons 1973). The plant has been referred to as the 'weed extraordinary' (Campbell 1963); such is its aggressive capabilities to infest large tracts of land and its inconspicuous nature. Infestations mainly occur on pastureland, although *N. trichotoma* is increasingly invading natural environments, such as National Parks and water catchment areas, which threatens native grassland and affects the habitats in these areas (Briese and Evans 1998).

Serrated tussock is believed to have arrived in Australia in the early 1900s. In 1935 serrated tussock was recorded at Yass, 55 km north-east of Canberra in southern New South Wales, where it was believed to have originated from seed in fodder imported during drought (Green 1956, McLaren *et al.* 1998). Approximately 4 ha was discovered at Broadmeadows, Victoria in 1954, where it was believed it had been growing for at least 20 years (Parsons 1973). By 1980 it had spread and occupied an area of 30 000 ha (Lane *et al.* 1980) and by 1998 occupied more than 130 000 ha of Victoria (McLaren *et al.* 1998). Serrated tussock is also present in Tasmania (Goninon 1998) and the Australian Capital Territory (Campbell and Vere 1995).

Various control measures have been implemented, including chemical, pasture competition, grazing management and manual control. Differences in results for some of these control measures have been noted (Miller 1995, Campbell and Nicol 2001), with particular regard to herbicide efficacy. As a biological control program is currently underway to investigate pathogens that show promise at controlling serrated tussock in Argentina (Briese *et al.* 2001), it is also necessary to identify any variation in the target plant in Australia to ensure that biological control agents selected will attack the full range of the plant in Australia. It is therefore important to understand, as fully as possible, the biology of the plant.

A systematic study was undertaken to investigate reports of phenotypic variation in *N. trichotoma* in Australia. For the first time, provenances of serrated tussock from various areas in Australia were grown under the same environmental conditions, with the progeny of these plants used in a germination trial. A brief overview of the trials will be given, with expanded details given in future articles.

Materials and methods

Morphology

Seeds were obtained from various provenances of serrated tussock from infested sites throughout Australia. Seeds were germinated in Petri dishes, transplanted into small peat moss pots (Jiffy pots) with a final transplantation, once seedling roots had established, to 200 mm diameter pots. Plants were grown in a polyhouse at the Keith Turnbull Research Institute (KTRI) at Frankston, Victoria, where they were subject to seasonal variation in natural daylight and temperature. Twenty-nine provenances were grown; two from the ACT, five from New South Wales (NSW), 21 from Victoria and one from Tasmania, giving 772 plants in total. Plants were rotated monthly. Height (maximum leaf length), diameter and circumference at base and half-height were measured at seven months in 1999 and 16 months after germination in 2000.

Data were analysed using restricted maximum likelihood (REML) models (Payne 2000). For each measurement, the effects of states and territories were divided into (i) an effect of Victoria versus all other states (NSW and Tasmania) and territories (the ACT) and (ii) an effect of NSW versus ACT versus Tasmania.