Biological control of serrated tussock (*Nassella trichotoma*): Is it worth pursuing?

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Summary

The potential for biological control of the weedy grass, *Nassella trichotoma*, in Australia is discussed in the light of recent surveys for pathogens in its native range and an improved understanding of the taxonomic relationships of *Nassella* to other Australian and South American genera of the tribe Stipeae. This information gives increased optimism that a suitable biological control agent or agents can be found. The type of research required to do this is briefly discussed.

Introduction

Serrated tussock (*Nassella trichotoma* (Nees) Arech.) is a perennial grass of southern South American origin that was accidentally introduced into Australia in the early 1900s and has since become a serious weed problem in south-eastern Australia (Campbell and Vere 1995). It has also become a weed in New Zealand, South Africa and the United States. Serrated tussock is highly invasive, producing large quantities of seed that are widely dispersed by wind and capable of forming dense infestations.

In Australia, the most seriously affected country, infestations occur mainly on pasture land, where the weed greatly reduces carrying capacity as it is unpalatable to stock. If forced to graze it, sheep lose weight and may die (Campbell and Vere 1995). Increasingly, serrated tussock is invading natural environments, in National Parks and water catchment areas, where it threatens native grasslands and modifies natural habitats. The most recent survey of its distribution in New South Wales (Jones and Vere 1998) found that serrated tussock occupies 880 000 ha, indicating continued spread in the past 20 years. Moreover, an additional 2 million ha have occasional plants present and can be considered at risk. In Victoria, infestations have increased to 130 000 ha with a potential range of 4 million ha (D. McLaren, personal communication). In Tasmania, the area infested is smaller (c. 1500 ha) but spreading (Goninon 1998). The economic losses attributed to serrated tussock are currently of the order of $40m per year in New South Wales (Jones and Vere 1998) and $5m per year in Victoria (Nicholson 1997).

Serrated tussock is a declared noxious weed in New South Wales, Victoria and Tasmania. Despite this, current control measures, revolving around the use of the herbicide flupropanate, have not prevented the continued spread of the weed (Campbell and Vere 1995). It is clear that other control options need to be investigated in order to develop a more effective management strategy for this weed. One such option is classical biological control; the introduction of a highly specific natural enemy(ies) from their native range that is capable of killing *N. trichotoma* plants or reducing their vigour, capacity for dispersal and ability to compete with native and pasture grasses.

The first survey of *N. trichotoma* was undertaken by Wells (1977) in Argentina, to assess the potential for its biological control in South Africa. He found that it was not an aggressive or weedy plant in its native range, due in part to the effects of natural enemies, including insects, fungi and rodents. A follow-up survey for insect natural enemies was made by Erb (1988), that identified 10 species of weevil and two moths. Unfortunately, while some species specialized on tussock grasses, all insects attacked a range of other grass species. Evans (1991) reviewed the literature on arthropod natural enemies of grassy weeds and concluded that there is an absence of records of host specific insects which may be due to the simplistic and uniform architecture of the Graminae which favours polyphagy and reduces the evolutionary pressures for monophagy. While Erb (1988) confirmed that 'the combination of insect and fungus attack can provide a control' of serrated tussock, South Africa did not pursue this work.

Wapshere (1990) examined the possibilities of controlling Australian grassy weeds biologically. He reported that, although no fungus had been recorded from *N. trichotoma*, several rust and smut fungi infected both members of the genus *Nassella* and the closely related *Stipa* in South America. This led him to conclude that biological control was unlikely for serrated tussock, because *Stipa* contains several native Australian species that are useful pasture grasses and the risk to them would preclude the introduction of any pathogen. In contrast, Evans (1991) assessed the potential of fungal pathogens as biological control agents of tropical grassy weeds and considered that, although there was still an urgent requirement for more basic research, the prospects appeared to be promising. Using itch grass (*Rottboellia cochinchinensis*) as a case study, he found that highly specific fungal pathogens occur on this grassy weed in its centre of origin (Evans 1995). Indeed, both the rust and smut species under evaluation proved to be too specific with clearly defined pathotype-biotype associations. Thus, it is probable that a suite of pathotypes will have to be selected in order to cover the range of weed biotypes present in the neotropical target area.

Nonetheless, the view that natural enemies of grasses are not sufficiently specific and that the risk to economically-important species of grass is too great to permit implementation of classical biological control remains a common one, and has discouraged such projects in Australia. This paper therefore re-examines the potential for biological control of *N. trichotoma*, in the light of an improved understanding of its taxonomic affinities and recent preliminary surveys for pathogen natural enemies in its native range.

Agent host-range and the taxonomic relationship of *Nassella* to other grasses

Given Wapshere's (1990) contention that *N. trichotoma* is 'probably too closely related to native *Stipa* spp. to allow the introduction of agents', it is pertinent to re-examine the taxonomic position of serrated tussock. Its relationship to other grass taxa has long been problematic, at times being placed within the large genus *Stipa* containing several hundred species in Eurasia, South America and Australia. The one point of agreement has been that serrated tussock belongs to the tribe Stipeae, though this tribe has variously been placed in the subfamilies Pooidae and Arundinoidae in different morphological classifications of the Poaceae (Clayton and Renvoize 1985, Watson *et al.* 1985).

Recent molecular phylogenetic evidence (Hsaio *et al.* 1995, Barker *et al.* 1995) suggests that Stipeae does not sit comfortably in either sub-family, though only Simon (1993) has erected a new subfamily (Stipoideae) for it. Simon (1993) listed only four genera within the tribe Stipeae that are present in Australia: *Nassella* containing six introduced species (McLaren *et al.* 1998), *Stipa* containing over 60 native species (Vickery *et al.* 1986), *Piptatherum* represented by a single species, *P. miliacea*, introduced as a pasture grass but not currently utilized widely, and *Piptochaetium*, represented by a single weedy species introduced from South America (Simon 1993).

Nassella has at various times been considered a subgenus of *Stipa* or a genus in its own right (Barkworth 1990), while *N. trichotoma* has been shifted between the two genera. *Nassella* was first revised by Parodi (1947) who excluded *N. trichotoma*, while Corti (1951) assigned it as the unique member of the section *Nassellopsis* within *Stipa*. This is the classification maintained today by some South American botanists (Zuloaga *et al.* 1994), whereas Australian taxonomists included it within the genus *Nassella* (Vickery and Jacobs 1980). In the most recent revision, Barkworth (1990) redefined the circumscription of the genus to include all species of Stipeae with strongly overlapping lemma margins and lemma apices that are fused into a crown. This expanded genus comprises 79 species including *N. trichotoma*, *N. neesiana* and 60 South American species formerly assigned to *Stipa*.

Barkworth and Everett's (1987) examination of the relationships of taxa within the Stipeae, including for the first time a comparison of Australian and non-Australian *Stipa* species, sheds most light on taxonomic position *vis a vis* biological control. They identified a number of monophyletic species-groups, including five endemic to Australia, covering the previously described genera (Figure 1). While South American species within *Stipa* were close to species of *Nassella*, both these groups were quite distinct from the Australian groups of *Stipa* (Figure 1). In fact, these differences are considered sufficient to warrant erecting a new genus, *Austrostipa* comprising 13 subgenera, to describe the Australian native taxa (Jacobs and Everett 1996). In addition, all three exotic species present in Australia that were assigned to *Stipa* have recently been placed in other genera; *S. brachychaeta* and *S. caudata* are now considered to belong to the Eurasian genus *Achnatherum* (Jacobs *et al.* 1995), while the recent South American invader, formerly known as *S. papposa*, has been renamed *Jarava plumosa* (Jacobs and Everett 1997). The stipoid genera present in Australia, as currently interpreted, are shown in Table 1.

Given the differences between Australian and American lineages, the relationships amongst South American species of the tribe Stipeae and the host range of pathogens found on them provides little evidence of any potential threat to Australian species. Nonetheless, it is clear that genera of the tribe Stipeae contain the most closely related grasses to the weedy *Nassella* species in Australia, and the first step in a biological control program will be to test any candidate pathogens against representatives of the subgenera of *Austrostipa*, before proceeding with a wider host-test list. Following the centrifugal phylogenetic testing sequence of Wapshere (1974), subsequent testing might involve species of the four introduced Stipeae genera represented in Australia (Table 1), followed by representatives of other tribes in the Arundinoideae and Pooideae. Given that there has been no previous introduction of a pathogen for the biological control of a grass, this list

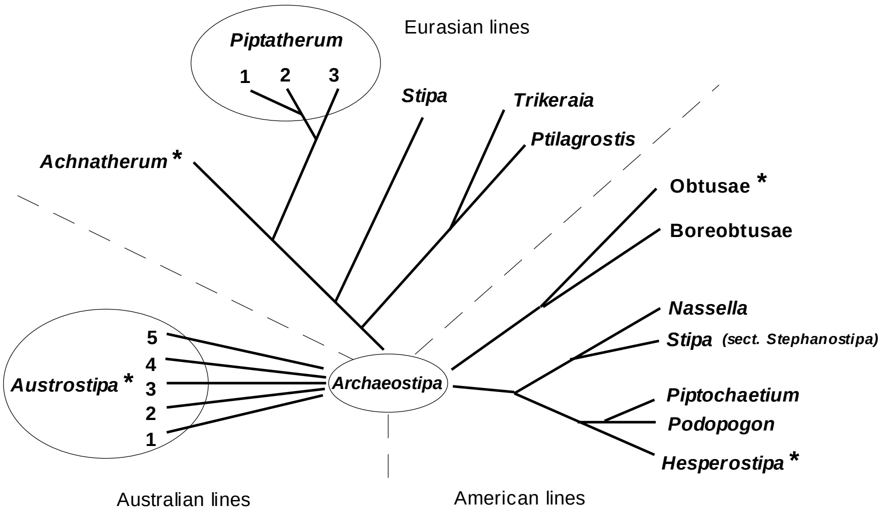

Figure. 1. The phylogenetic relationships of monophyletic taxa in the tribe Stipeae. Numbers indicate where several monophyletic groups occur within one genus, and asterisks indicate those genera or monophyletic groups containing species previously assigned to the genus *Stipa*. There are several other groups within the American lines of *Stipa*. Figure modified from Barkworth and Everett (1987).

Table 1. Genera of the tribe Stipeae present in Australia that would need to be considered in any host-testing program for biological control agents of serrated tussock.

Genus	No. of species	Origin	Status
Austrostipa	62	Australia	native taxon with several useful pasture and several weedy species
Nassella	6	South America	declared noxious weeds or potentially weedy
Achnatherum	2	South America	potentially serious weeds
Jarava	1	South America	localized minor weed
Piptochaetium	1	South America	localized minor weed
Piptatherum	1	Eurasia	minor pasture grass and used in mine reclamation

would probably need to be conservative and include selected grasses from even-more distantly related taxa to assure all parties with interests in economically important grasses that any introduction was safe. The final test list would therefore be quite long and would undoubtedly need to include cereal, pasture, turf and native species.

Recent South American surveys

Biological control agents are, by definition, highly adapted to their host plant, and the chances of finding such an organism will be greatest near the centre of diversification of the target weed genus. The distribution of *Nassella* species (Barkworth 1990), shows clearly that this genus is centred in temperate South America, and Wells (1977) postulated that *N. trichotoma* originated in the Sierra de Ventana region of Argentina. Hence, in 1995 CSIRO Entomology engaged IIBC to survey populations of *N. trichotoma* in Argentina for pathogens that may have potential as biocontrol agents. The main survey of one week's duration was undertaken in March 1995 (Evans and Ellison 1995), followed up by observations made in conjunction with surveys on other weed species during November 1995 and April 1996 (Evans 1996). Collections were made at 17 sites in central and western Argentina in March 1995 and at one further site in north western Argentina in April 1997 (Figure 2). Nine species of fungi were identified from serrated tussock plants, and these are listed in Table 2.

Based on the initial survey, Evans and Ellison (1995) concurred with the conclusion of Wells (1977) that, in non-agricultural or natural ecosystems in Argentina, *N. trichotoma* appears to be part of the indigenous flora rather than an invader and that fungal pathogens would seem to play a role in controlling *Nassella* populations.

Some of these pathogens have very broad host and geographic ranges. For example, the smut *Ustilago hypodytes* is already known from a range of Australian stipoid grasses. Others appear to be much more specific. The two pathogens of

Table 2. Fungi collected by H.C. Evans and C.A. Ellison during surveys of *Nassella trichotoma* in Argentina in 1995 and 1996.

Fungal pathogen	No. of sites (out of 18)	Symptoms
Basidiomycota: Basidiomycetes: Stereales		
Corticium sp.[A]	11	Infected plants having snow-white mycelia at stem / root interface, turning to grey on centres of dead or dying tussocks
Basidiomycota: Teliomycetes: Uredinales		
Puccinia nas(s)ellae[B] Arth. and Holw.	2	Rust pustules within tightly rolled leaves on plants suffering dieback. More common on young plants at one site
Uredo sp.	1	
Basidiomycota: Ustomycetes: Ustilaginales		
Ustilago hypodytes (Schlecht.) Fr.	1	Inflorescences replaced by smutted heads
Deuteromycotina: Coelomycetes		
Aschochyta leptospora var. *variispora* Punith.	2	Associated with leaf chlorosis and subsequent necrosis
Stagonospora sp.[A]	7	Associated with leaf chlorosis and subsequent dieback of leaves and flowering stems
Hendersonula sp.[A]	1	
Septoria sp.[A]	1	
Deuteromycotina: Agonomycetes		
Rhizoctonia sp.	1	Leaf bases colonized by network of dark brown hyphae associated with chlorosis

[A] All probably represent undescribed species (*fide* IMI Reports). [B] Orthographic error; in this publication, and continued in subsequent ones (Greene and Cummins 1958), the host genus was listed as *Nasella*, hence the specific epithet *nasellae.*

greatest immediate interest for biological control are *Corticium* sp. and *Puccinia nas(s)ellae. Corticium* sp. was the most common fungus found during the field surveys, forming a mycelial mat in the base of the tussocks and being associated with severe tussock decline at 11 of the 18 sites sampled (Figure 2). Tussock grasses from other species that occurred in the same area were not affected with these symptoms, suggesting that there may be a high degree of specificity. There has been no taxonomic treatment of this species outside Europe and North America and it remains undescribed. Rust fungi have already been successfully used in a number of biological control projects (Julien 1992), and one such species, provisionally identified as *P. nas(s)ellae,* was found heavily attacking the leaves of a number of serrated tussock populations at one location in northwestern Argentina (Figure 2). According to Greene and Cummins (1958) and Cummins (1971), *P. nas(s)ellae* is known only from other species of *Nassella* (following Barkworth's (1990) revision of the genus). However, the form collected has smaller urediniospores (16–18 × 19–20 mm) than *P. nas(s)ellae* sensu stricto (23–26 × 26–30 mm according to Cummins 1971) and could well be an undescribed taxon (R. Treu personal communication). Aecia are unknown (Green and Cummins 1958, Lindquist 1982), and thus it has not been proven if the rust is autoecious or heteroecious. This can only be established by detailed field studies.

Discussion

The most recent taxonomic studies have highlighted the polyphyletic origins of the genus *Stipa.* They indicate that, rather than serrated tussock belonging to a large cosmopolitan genus *Stipa,* there are several genera involved, with *Austrostipa* present in Australia, and an enlarged *Nassella,* including *N. trichotoma, N. neesiana* and many South American species formerly in *Stipa,* centred in South America. The likely Gondwanan origins of the Stipeae (Barkworth and Everett 1987) suggest a long period of separate evolution of the taxa and their associated mycofloras. The difference in mycofloras of South American and Australian stipoid taxa is indicated by the fact that, based on collection material held in New South Wales and Victoria, none of the 27 species of fungi known from *Austrostipa* in Australia have been recorded on introduced *Nassella.* Moreover, none of the rusts found on South American taxa are known from Australian ones, while *Puccinia flavescentis,* common on *Austrostipa* spp. is not recorded from South America (Cummins 1971).

In addition, revision of the stipoid taxa suggests that some pathogens from *Nassella* may be even more specific than previously thought, as none of the rusts described by Cummins (1971) from *Nassella* spp. had recorded host ranges outside the tribe Stipeae, and five of the nine species only had hosts within the genus itself. Indeed, there is now taxonomic evidence for the existence of host specific forms (races, strains, varieties) within *Puccinia nas(s)ellae*. Lindquist (1982), in his monograph of Argentinian rusts, listed several hosts for *P. nas(s)ellae,* sensu Arthur and Holway (Greene and Cummins 1958): *N. caespitosa, N. chilensis* and '*Stipa*' sp., but erected a new variety for the rust on '*Stipa*' *neesiana, P. nas(s)ellae*

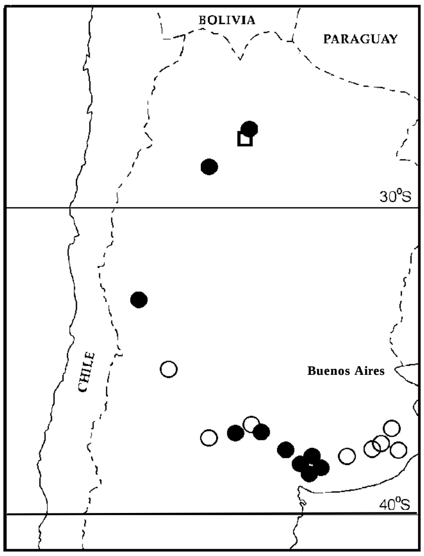

Figure 2. Collection sites of fungi found on *Nassella trichotoma* in Argentina during 1995–96. Closed circles = sites with heavy attack by *Corticium* sp. Open squares = sites with heavy attack by *Puccinia* sp.

var. *platensis.* Surprisingly, *N. trichotoma* was not included as a host of this, or any other rust species, in Argentina (Lindquist 1982).

In fact, prior to the current surveys, no pathogens had previously been recorded from *N. trichotoma.* If nine species were recorded during such a brief investigation, it is highly likely that further fungal pathogens would be collected should more widespread surveys over a full range of seasons be possible. The fact that the Australian and American taxa are less closely related than previously thought, the indication that these grasses harbour a rich and incompletely known mycoflora and the apparently high degree of specificity in the known range of some pathogens collected from them gives greater optimism for finding a suitable biological control agent. While accepting that any biological control project carries a risk of failure, there is ample justification to pursue the detailed studies needed in South America to accurately determine the potential of pathogens for control of serrated tussock. Such a project would also provide the opportunity to investigate the potential of pathogens to control the related Chilean needle grass, *N. neesiana*, an emerging weed problem in south-eastern Australia.

These studies should aim to:

- initially carry out detailed surveys throughout the range of *N. trichotoma* and *N. neesiana* (concentrating on those areas that are ecoclimatically most similar to infested areas of Australia) for pathogens with biological control potential and select a short-list of pathogens for more detailed study,
- investigate the specificity of these pathogens, starting with *Puccinia nas(s)ellae* and *Corticium* sp., against key Australian species such as *Austrostipa* to determine whether it is safe to proceed with more detailed studies, and if so,
- study their epidemiology and impact on the population dynamics of selected field populations of *N. trichotoma* and *N. neesiana*, and concurrently
- develop culture methods for them in the laboratory and study their biology, life-cycle and virulence against Australian forms of the weeds.

The end-point would be the identification of one or more pathogens with demonstrated potential for biological control, a well-understood biology and having proven specificity against key grass species. The project would then be ready to enter a second phase aimed at quarantine clearance and eventual release as part of the management strategy for weedy *Nassella* species in Australia.

Acknowledgments

We would like to thank Joy Everett, Surrey Jacobs, David McLaren and Louise Morin for reviewing earlier versions of this manuscript. Michael Priest (NSW Agriculture) and Ian Pascoe (Agriculture Victoria) provided lists of pathogens collected from *Stipa* (=*Austrostipa*) and *Nassella* in Australia. Bob Sproule and Gratton Wilson of the NSW Serrated Tussock Working Group organized the consortium of funding to support the South American surveys, which included the Cooma-Monaro Shire Council, NSW National Parks and Wildlife Service, NSW Noxious Weeds Advisory Council, and the Snowy Mountains Authority.

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