# Investigations into micro-morphological discrimination of vegetative Mexican feather grass, *Nassella tenuissima* from Serrated tussock, *Nassella trichotoma* and other stipoid species.

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**Summary** Mexican feather grass (*Nassella tenuissima*) is a nationally prohibited weed that has been sold via the nursery trade across Australia. MFG has been described as being a potentially more serious weed to Australia than serrated tussock, that is currently costing Australia in excess of $50 million annually. The confusion over plant names and plant taxonomy in plants being sold via the nursery trade has resulted in the need of a technique to definitively identify vegetative MFG grass plants from other stipoid grass species in the absence of flowering plant parts. This paper reports on progress towards developing micro-morphological techniques of using characters of the vegetative parts such as sheath, leaf blade and ligule to differentiate MFG grass from serrated tussock and other stipoid species.

**Keywords** *Nassella tenuissima*, *Nassella trichotoma*, stipoid grasses, micromorphological techniques, sheath, ligule, leaf blade.

## INTRODUCTION

Mexican feather grass (MFG) *(Nassella tenuissima* (Trin.) Barkworth), is an unpalatable exotic stipoid grass threatening grazing industries and indigenous grasslands of Australia. Native to New Mexico, Texas, Argentina and Chile, it has also been cultivated in Australia, New Zealand and South Africa (Jacobs *et al.* 1998). MFG is closely related to other exotic stipoid grasses, including Serrated tussock (*Nassella trichotoma* (Nees) Arech.) and Chilean Needle grass (*Nassella neesiana* Trin. & Rupr.), both of which have been determined as "Weeds of National Significance" to Australia (Thorp and Lynch 2000). Serrated tussock is a serious pasture and environmental weed in temperate Australia, costing the Australian grazing industry an estimated $45 million annually through loss of productivity and control (Cowan *et al*. 2007). MFG is less palatable than serrated tussock and has the potential to invade nearly six times as much land in Australia as serrated tussock (McLaren *et al.* 1999).

In the 1990s, native grasses became very popular as ornamental plantings in Australia and native grasses such as Poa tussock, *Poa labillardierei* were used extensively as a low maintenance, attractive groundcover along road and freeway verges. The popularity of ornamental grasses has resulted in the unfortunate introduction and sale of MFG via the Australian nursery trade where it has been sold under a range of names including Angel's Hair, Elegant spear grass, Ponytail grass, Texas tussock grass, White tussock, and varying botanical names including *Stipa tenaccissima* and *Stipa tenuissima* (McLaren *et al.* 1999). MFG was originally imported into Australia as *Stipa tenuissima* in 1996. At this time the *Stipa* genus was a permitted import through the Australian Quarantine and Inspection Service (AQIS). This loophole has now closed and Australia has adopted a permitted species system that only allows entry of AQIS assessed plant species into Australia. All *Nassella* species are now prohibited from importation to Australia. The confusion of MFG identification has resulted in it entering the commercial nursery trade in Victoria, South Australia, Queensland and the ACT. The importance of being able to distinguish MFG in its vegetative state from other commercially grown grasses is critical in preventing its sale and spread in Australia. A micro-morphological assessment of MFG and five other Poaceae species has been undertaken. This paper reports on a micro-morphological comparison of taxonomic characters of vegetative MFG and its close taxonomic relative, serrated tussock.

## MATERIALS AND METHODS

**Plant specimens** MFG and serrated tussock plants used in this study were collected from five different geographic accessions. MFG plants were recovered from private properties in Sunbury (37º 34', 144º 42'), Ferntree Gully (37º 53', 45º 17'), Tarneit (37º 51', 144º 39') and Footscray (37º 47', 144º 54') by Department of

Primary Industries (DPI) officers in 2009. Serrated tussock plants were collected from Diggers Rest, Victoria (-37º 37', 144º 44'), (-37º 42', 144º 39') and Armidale, NSW (-30º 18', +151º 21'), (-30º 21', +151º 22'), in 2003. In addition, specimens of MFG and serrated tussock were grown from seed collected from commercially purchased plants in 2005. In total, five specimens of each species from each location were analysed. Plants were potted in 10-25 cm diameter pots with standard potting mix and grown in a glasshouse with natural lighting and controlled irrigation at DPI Frankston.

**Morphological analysis** For each *Nassella* species, at least ten leaves were selected for examination from each of the five plant specimens examined. For consistency, observations were made on healthy, non-flag leaf blades from an area midway between the blade apex and the ligule. Forty-two vegetative characters were observed, of which eight were measured and one was counted (Table 1). Leaf dimensions were recorded by measuring blade length, sheath length and blade width at the widest point. All length and width measurements were recorded microscopically, except for blade and sheath length which were recorded with a standard measuring ruler. Trichome length, trichome angle (º) of growth to the abaxial leaf surface, and the density of trichomes per 200 $\mu m^2$ was also recorded. Ligule length was measured from the base of the collar to the tip of the ligule and the breath of the ligule was taken at the widest point. At least ten measurements were taken for each character (excluding sheath, blade and ligule length) per leaf blade studied. Each measured character was averaged for the 5 plant specimens and recorded as the mean ± 1SD. Range is indicated within brackets (Table 1).

**Microscopic assessment** Micro-morphological observations were made with an Olympus SZX-16 stereo microscope with a zoom ratio of 16.4:1 (0.7×-11.5× magnification). Images were documented with an Olympus UC30 camera, and processed with AnalySIS FIVE, image analysis software.

## RESULTS

**Identification** Following micro-morphological examination, several distinctive features (Table 1) were apparent between MFG and serrated tussock;

**Leaf sheaths** In both *Nassella* species, the sheaths are tight around the base of the leaves, shallot-like, non-auriculate, round in cross section, lightly striate and possess oral seta (base of ligule). Sheaths of MFG are moderately to densely pubescent-hirsute, whilst those of serrated tussock are only marginally puberulous and feel fairly smooth, except for the sparsely hirsute margins. In both species, sheaths are bearded and glabrous towards the base, with a distinctive purple-tinged colour in MFG, while sheaths of serrated tussock appear beige-white. Sheaths of MFG are approximately double in length (11±3 cm) to those of serrated tussock (5±0.4 cm).

**Leaf blade** Both species have numerous convolute-filiform, straight or curving (at maturity) blades with intravaginal innovations. Leaves are tightly rolled and appear cylindrical in cross section. Blade diameter (rolled) ranges from 0.3-0.5 mm for both species, whilst blade height is to 50 and 70 cm, for serrated tussock and MFG, respectively. Leaves of both species are antrorsely and retrorsely serrated, bristly and coriaceous in texture. Blades of serrated tussock are harshly scabrid with a dense coverage of 2.7±0.3 trichomes per 200 $\mu m^2$, whilst those of MFG are scabrous with a moderate trichome density of 1.7±0.1 on the abaxial leaf surface, thus giving it a less setaceous feel. Both MFG and serrated tusssock have tooth-like serrations (at approximately 33º to the abaxial leaf surface) along the full length of the blade margin. In both species, the aristate blade apex is bleached, straw coloured. However, Serrated tussock has a characteristic brown strip marking on the blade apex not seen in MFG.

**Ligule** The ligule of serrated tussock and MFG is membranous, 1-2 mm long, obtuse, moderately to strongly involute and chartaceous in nature. The ligule of MFG is minutely serrated (20-31 µm), whereas the ligule of serrated tussock is pubescent to hirsute (30-54 µm). The ligule apex of MFG is translucent, becoming acute, not hairy and with an erose-jagged incision, whilst the ligule apex of serrated tussock is opaque, entire, rounded, occasionally retuse, hairy and sometimes yellow, particularly on the outer leaves.

**Table 1.** Micro-morphological characteristics of MFG and Serrated tussock.

| **Vegetative characters** | MFG | Serrated tussock |
|---|---|---|
| *Leaf sheath looseness* | tight | tight, closely packed |
| *Sheath margins* | glabrous to moderately hirsute | glabrous to sparsely hirsute |
| *Sheath venation* | lightly striate | lightly striate |
| *Sheath roughness* | antrorsely and retrorsely scaberulous | fairly smooth & minutely scabridulous |
| *Sheath surface indumentum* | moderately to densely pubescent/hirsute, glabrous towards base, sheath base bearded | marginally puberulous, glabrous towards base, sheath base bearded |
| *Oral hairs- beside ligule* | a few scanty bristles (oral seta) | a few scanty bristles (oral seta) |
| *Sheath colour* | purple tinged, beige at base | beige-white, never purple |
| *Sheath length (cm)* | 11±3 (4.5-18) | 5±0.4 (2-9) |
| *Collar* | glabrous | glabrous |
| *Auricles* | non-auriculate | non-auriculate |
| *Basal innovations* | intravaginal | intravaginal |
| *Leaf blade outline* | filiform, aciculate | filiform, aciculate |
| *Leaf blade attachment* | mostly basal | mostly basal |
| *Blade vernation* | convolute | convolute |
| *Blade cross section* | terete-circular | terete-circular |
| *Blade length (cm)* | 28±11 (3.5-70) | 22±4.3 (2.5-50) |
| *Blade width (µm)* | 417±24 (347-521) | 408±11 (297-511) |
| *Blade texture* | bristly, coriaceous | coriaceous, very bristly |
| *Blade venation* | non-striate | non-striate |
| *Blade roughness* | scabrous | harshly scabrid, scabrous |
| *Blade indumentum- abaxially* | serrated-tooth like prickles | serrated-hirsute & tooth like prickles |
| *Blade indumentum- adaxially* | setaceous, hirsute | setaceous, hirsute |
| *Blade trichome density* | moderate | moderate to dense |
| *Trichome density- per 200 µm$^2$* | 1.7±0.1 (1.4-2.2) | 2.7±0.3 (2.1-3.4) |
| *Trichome projection* | antrorse & retrorse | antrorse & retrorse |
| *Trichome projection (angle º)* | 33±1.3 (27-40) | 33±2.6 (27-42) |
| *Trichome length (µm)* | 58±6.4 (38-69) | 66±3.8 (60-78) |
| *Blade margins* | serrated-tooth like, more so closed | serrated-tooth like, more so open |
| *Blade apex- shape* | aristate, filiform, linear | aristate, filiform, linear |
| *Blade apex- colour* | fawn, bleached | fawn with brown marking/streak |
| *Blade apex- vesture* | small serrations | small serrations/hirsute |
| *Ligule type* | membranous, hyaline | membranous, papery |
| *Ligule length (µm)* | 1565±147 (1139-2065) | 1361±61 (1097-1837) |
| *Ligule width (µm)* | 385±39 (260-618) | 334±14 (265-380) |
| *Ligule colour* | translucent on top, opaque towards base | opaque-whitish |
| *Ligule- abaxial indumentum* | minutely serrated, prickle toothed | pubescent to hirsute |
| *Ligule apex- incision* | erose, jagged, not hairy | entire, rounded, occasionally retuse or emarginate, hairy |
| *Ligule apex- shape* | obtuse, becoming acute, long | obtuse, long |
| *Ligule apex- colour* | white, clear | white to yellow (on outer leaves) |
| *Ligule margins* | entire, strongly involute or terete, marginal whiskers | moderately involute, very bristly |
| *Ligule trichome density* | moderate | moderate to dense |
| *Ligule trichome length (µm)* | 26±1.9 (20-31) | 39±3 (30-54) |

To facilitate the identification of vegetative specimens of MFG, serrated tussock and other Poaceae the following key was devised:

1. Leaf blades tightly inrolled (0.3-0.5 mm), densely tufted, basally disposed, filiform (needle like) and serrated……. ...2
1. Leaf blades usually flat or loosely rolled and not serrated……...*Poa* spp., *Stipa* spp.
2. Leaf sheaths moderately to densely pubescent, purple-tinged, to 18 cm long……... 3
2. Leaf sheaths smooth to marginally puberulous, beige-white, to 10 cm long……...4
3. Leaf blades scabrous (1.4-2.2 trichomes per 200 $\mu m^2$), to 70 cm long, blade apex bleached, never brown ……....MFG
3. Leaf blades scabrous, to 70 cm long, blade apex green…….5
4. Leaf blades scabrid (2.1-3.4 trichomes per 200 $\mu m^2$), to 50 cm long, blade apex bleached with brown marking ……serrated tussock
4. Leaf blades scabrid, to 50 cm long, blade apex green……6
5. Ligule membranous-hyaline, obtuse-acute, minutely serrated (26±1.9 µm), apex translucent and erose, not hairy……..MFG
6. Ligule membranous, opaque-white, pubescent to hirsute (39±3 µm), apex obtuse, entire, rounded and hairy.....serrated tussock

## DISCUSSION

The similarities in vegetative taxonomy of MFG to other commercially grown grass species has made definitive grass identification problematic. Given the legal implications of commercial nurseries potentially selling noxious weeds, it is essential that fast, reliable techniques are developed for differentiating MFG in its vegetative state from other commercially sold species. Previously, large numbers of plant samples have been seized from commercial operators as they were suspected of being MFG. Generally these vegetative stipoid grass samples could not be definitively identified because the standard taxonomy is based on fertile and not vegetative characters. This has necessitated housing and growing these plants in secure glasshouses until they become reproductive enabling identification. This is both costly to Governments and potentially a huge impost on the commercial operators if MFG identification proves false. The assessment of micro-morphological taxonomy of vegetative MFG and serrated tussock has revealed several characters that can distinguish MFG from other stipoid grass species. This has enabled the development of a short key that highlights: (1) trichome number per 200 $\mu m^2$ of the leaf surface, (2) the leaf blade apex colour (3) the ligule incision and hair length (µm) and (4) the colour and length of the MFG leaf sheath (cm) that can be used. This research has shown that there are several distinguishing features of MFG that can separate this species from serrated tussock and will also differentiate this species from other Poaceae species during the vegetative stage. Additional research is also being undertaken to assess the capacity of using molecular techniques in differentiating stipoid grass DNA. Combining the molecular and micro-morphological taxonomy should provide compelling evidence for future MFG identification.

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