Investigations into natural serrated tussock (*Nassella trichotoma*) die-back events observed in Tasmania and NSW

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Summary Two major serrated tussock die-back events occurred in Australia during 2009 and 2011. The pathogen, *Fusarium oxysporum* has been identified infecting serrated tussock plants at both locations and was implicated in the die-back. *Fusarium oxysporum* varieties can be pathogenic and have been used as classical biological control agents in the past. Previous glasshouse trials failed to confirm *F. oxysporum* as the causal agent of the serrated tussock die-back. This project investigated whether field collected affected tiller pieces could transmit disease symptoms to healthy serrated tussock plants in a pot trial. Significant decline in serrated tussock health was observed in treated compared to untreated serrated tussock plants. A later visit to the NSW affected property identified many serrated tussock plants showing white mycelium fungal growth from their crowns and lower shoots and significant nematode root damage. Potential reasons for the observed serrated tussock die-back are discussed.

INTRODUCTION

Serrated tussock (*Nassella trichotoma* Trin. & Rupr Barkworth) is causing huge agricultural and environmental impacts and is classified as Weed of National Significance in Australia (Thorp and Lynch, 2000). The recent identification of herbicide resistance to the most popular herbicide (flupropanate) for serrated tussock control highlights the need to identify new control tools for this debilitating noxious weed (McLaren *et al*. 2010).

A six year project assessing natural enemies for serrated tussock in its countries of origin failed to identify a suitable biological control agent (Anderson *et al*. 2006). Large patches of dense serrated tussock were found dying back for no apparent reason near Hobart in Tasmania during 2009 and near Lake Bathurst in NSW during 2011. In both cases, discussions with landowners suggested no herbicides had been applied to the regions in question. Samples of symptomatic plants were collected from both these sites and sent to the Victorian Department of Primary Industries Plant Health diagnostic laboratory for pathogen assessment. *Fusarium oxysporum* (Schlecht. emend. Snyder & Hansen) and several other pathogens were identified from these samples with *F. oxysporum* suspected of being a likely candidate for the observed serrated tussock die-back (James Cunnington *pers comm.*).

Fusarium oxysporum is a ubiquitous soil pathogen found all around the world containing many pathogenic and non pathogenic strains (Nelson *et al*. 1983). It can be an extremely damaging pathogen and there are many examples of biocontrol projects to contain *F. oxysporum* outbreaks on beneficial species (Fravel *et al*. 2003). However, some strains can be quite host specific and it has been previously used for classical biological control of weed species such as leafy spurge (*Euphorbia elula*) (Caesar *et al*. 1999), sicklepods (*Senna obtusifolia*) (Boyette *et al*. 1993), *Striga hermaonthica* (Ciotola *et al*. 1994) among several other species. *Fusarium oxysporum* has been also recovered from serrated tussock seeds in a soil seed bank trial and tests showed that this strain was virulent to serrated tussock seedlings but was not host specific or damaging to mature serrated tussock plants (Casonato 2003).

Fusarium oxysporum collected from Hobart in Tasmania and Lake Bathurst in NSW has been assessed for host specificity and virulence against 10 populations of serrated tussock from around Australia and five important agricultural and five indigenous grass species. Results from this experiment showed that *F. oxysporum* strains from both Tasmania and NSW did not have a significant impact on host plant survival (Cowan 2011). Ongoing discussions with the beef farmer, Hans Kazmaeir at Bathurst Lake suggested that the serrated tussock populations were continuing to be selectively impacted by an apparent pathogen. This project investigated whether field affected tiller pieces from affected serrated tussock plants collected from Bathurst Lake in NSW could transmit disease symptoms to healthy serrated tussock plants.

MATERIALS AND METHODS

Serrated tussock plants from Bathurst Lake showing symptoms of pathogen attack were collected and dispatched to DPI during December 2011 and were split into tillers with original soil still attached to their roots. The tillers were planted (with their soil) into pots containing mature previously untreated control serrated tussock plants sourced from 10 different serrated tussock populations collected from around Australia (**Victoria** – Little River, Mt Wallace, Werribee, Rowsley Valley, St Albans; **NSW** – Armidale, Goulburn, Abercombie, Bathurst Lake; **Tasmania** – Rokeby). The transplanted serrated tussock plants had their leaves trimmed (to help survival of transplanted tillers) after sowing. Treated serrated tusssock were compared to untreated control plants collected from the Rowsley Valley and St Albans populations kept under equivalent conditions in a separate glasshouse. Control plants also received tiller pieces transplanted from untreated serrated tussock plants. Serrated tussock plants were periodically assessed for damage using a 0–9 scale (0=healthy, 9=dead). A serrated tussock plant from each of the ten treated provenances was allocated to a bench and arranged randomly. Three benches provided the block replication. Due to serrated tussock availability, untreated controls were restricted to the Rowsley Valley and St. Albans populations. Two plants of each of the two untreated control plants were allocated to a bench and arranged randomly. Three benches provided the block replication. Plants within block were re-arranged at random each fortnight. Glasshouses were maintained at a constant 20–25°C and 60–80% humidity. Plant samples showing disease symptoms were sent to the Department of Primary Industries for pathogen and nematode assessment including molecular identification for pathogen species.

RESULTS

The effects of Bathurst Lake serrated tussock tiller addition treatments on ten untreated serrated tussock populations were compared to control serrated tussock populations using a two way analysis of variance (Payne *et al.* 2009) (Table 1). This showed significant impacts on treated compared to untreated plants and also significant differences within serrated tussock populations in the treated plants. Treated plants showed symptoms such as bright reddening of tillers followed by senesce and black spotting within the tillers 51 days after treatment application. Control plants remained green.

Results showing the average damage score and standard errors for the different serrated tussock populations treated are shown in Figure 1. The Victorian serrated tussock population sourced from Little River had the greatest damage score while the NSW serrated tussock populations from Abercombie, Armidale and Bathurst Lake had the least damaged compared to the untreated controls. A plant showing disease symptoms was sent to DPI Knoxfield for pathogen assessment by the Plant Health Services Section. The roots and crown samples were positive to *F. oxysporum* while the shoots were attacked by an *Epicoccum* sp.

This result prompted a follow-up visit to the NSW property where many serrated tussock plants were observed with white fungal masses in their crowns This was confirmed as *Rhizoctonia* sp. that are known pathogens of crops such as wheat, tomatoes and cucumbers among many more species (Gonzalez 2006). The roots and crowns were again infected by *F. oxysporm* while a nematode assessment identified 1,613 stubby-root nematode (*Paratrichodorus* sp.) and 93 spiral (*Rotylenchus* sp.) in 200 ml of soil surrounding the field collected serrated tussock roots. Stubby-root nematode feed externally on plant roots and a generalist feeders attacking a range of grass species. The density of stubby and spiral nematodes were considered very high and likely to be responsible and/or be contributing to the observed serrated tussock symptoms at the Bathurst Lake site.

Table 1. Two way analysis of variance – serrated tussock tiller treatments vs populations.

Source of Variation	d.f	s.s.	m.s.	v.r.	F pr.
Tiller treatments	1	57	57	31	<.001
Serrated tussock populations	11	91	8	4	<.001
Residual	35	65	2		
Total	47	213			

DISCUSSION

Unintended introductions of pathogens with significant weed biological control impacts are not uncommon in Australia. Some examples include biological control of *Hypericum androsaemum* with the rust *Melampsora hypericorum* (McLaren *et al.* 1997); *Rubus fruiticosis* with the rust fungus *Phragmidium violaceum* (Bruzzese and Field 1984); *Sporobolus fertilis* with the fungus, *Nigrospora oryzae* (Ramasamy *et al.* 2007). Serrated tussock plants dying for no apparent reason have been identified in both Tasmania and NSW during 2009 and 2011 respectively suggesting that a natural biocontrol process was tacking place. A range of organisms including *F. oxysporum, Alternaria* sp., *Rhizoctonia* sp., *Mucor* sp., *Phytophthora cryptogea* and the nematodes, *Paratrichodorus* sp. and *Rotylenchus* sp. were identified as possible causal agents (Cowan 2011). *Fusarium oxysporum* appeared the most likely candidate for causing selective die-back of serrated tussock populations from the Tasmania and NSW locations and was targeted for virulence and host

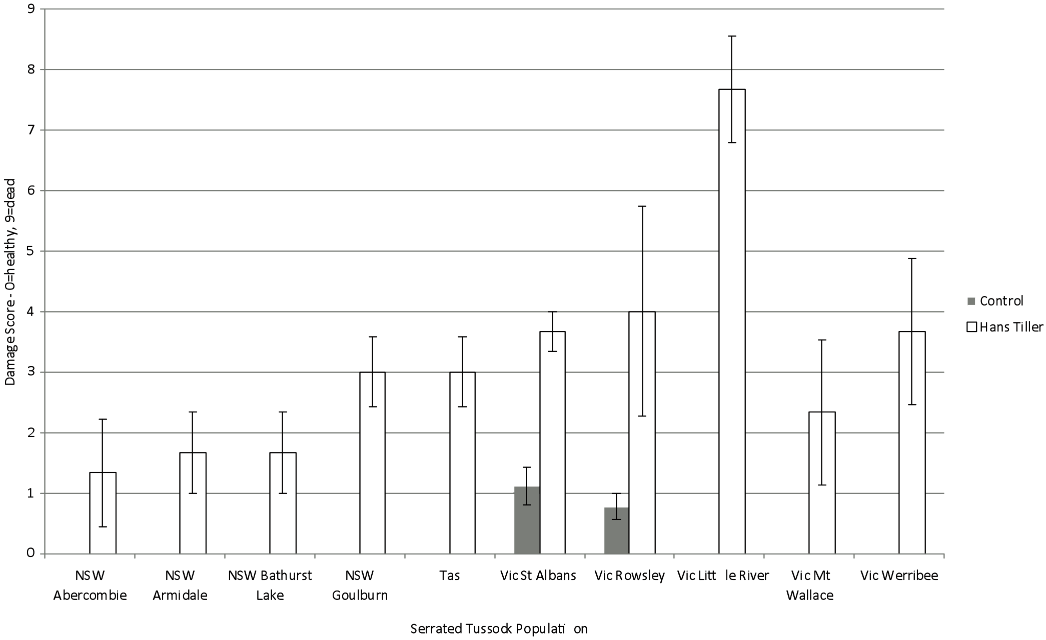

Figure 1. Effect of pathogen tiller transplants on serrated tussock damage scores 51 days after tiller transplants.

specificity testing but failed to show any significant responses (Cowan 2011).

This current study has shown that when field collected tillers from Lake Bathurst (showing die-back symptoms) were planted in pots with untreated serrated tussock plants it resulted in significant die-back of treated plants. A diagnostic pathogen assessment of one of the infected serrated tussock plants from this experiment revealed significant *F. oxysporum* from the roots and crown. Black pathogen lesions were also identified on the leaves caused by *Epicoccum* sp. *Epicoccum* sp. are mostly recorded as saprophytes but are also known to be parasitic on apple and millet (Kortekamp 1997).

The actions of a grazer and a pathogen have often been linked to spectacularly successful biological control programs. The success of the Prickly pear cactus (*Opuntia stricta*) biological control program in Australia was due largely to the combined actions of cactus cladode eating *Cactoblastis cactorum* larvae and an assortment of fungi including *Fusarium* spp. (Dodd 1940). The success of biological control of leafy spurge, *Euphorbia esulalvirgata,* in the USA has been attributed to the combined actions of the flea beetles, *Aphthona* spp. creating entry points for soil fungi such as *Fusarium oxysporum, Rhizoctonia* sp. and *Pythium* spp. (Caesar 2003). The recent discovery of nematode damage to the serrated tussock roots collected from Bathurst Lake may provide an important clue to the observed serrated tussock mortality taking place at the property in NSW. We hypothesize that the damage caused by the nematodes may be enabling soil borne pathogens such as *F. oxysporum* and *Rhizoctonia* sp. to gain access to serrated tussock roots causing the observed die-back. The land owners observations were that the die-back was also affecting a native grass species (*Austrostipa scabra*) while other native grasses were unaffected. The nematodes, *Paratrichodorus* sp. and *Rotylenchus* sp. are reportedly generalists but other exotic beneficial grasses on the property didn't appear to be affected.

This experiment also showed considerable variation in damage scores between serrated tussock provenances suggesting that their may be some genetic variation between serrated tussock populations in Australia. Seona Casonato assessed the taxonomy and variation of 36 serrated tussock populations across Australia (Casonato 2003). She determined that taxonomically and molecularly they were all serrated tussock but populations behaved differently in relation to seed germination, growth and flowering. This trial also indicates some variation in serrated tussock population susceptibility to possible pathogen/nematode attack.

Additional investigation into the relationships of serrated tussock to the nematodes – *Paratrichodorus* sp. and *Rotylenchus* sp. and the pathogens *F. oxysporum* and *Rhizoctonia* sp. could potentially provide some important information that could help land managers selectively control this debilitating weed. We would recommend conducting similar studies to Caesar (2003) who investigated the relationships of the flea beetle *Aphthona* spp and the soil pathogens *F. oxysporum* and *Rhizoctonia* sp. in the successful biological control of leafy spurge in North America.

ACKNOWLEDGMENTS

The authors would like to acknowledge Meat and Livestock Association for supplying the resources to enable this investigation to proceed. We also thank the landowner from the Bathurst Lake property in NSW, Hans Kazmeir for his interest, support and important observations on serrated tussock during this project.

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