WILL AUSTRALIAN ENDEMIC PATHOGENS WEAKEN THE MIGHT OF GIANT RAT'S TAIL (GRT) GRASS?

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ABSTRACT

Sporobolus is a genus containing 198 grass species from the tropics and subtropics, including Africa, temperate and tropical Asia, Oceania, North and South America. In Australia, 18 species are endemic, and a further six are naturalised weeds. Giant rat's tail grass (GRT) and the other introduced weedy *Sporobolus* grasses are unpalatable, perennial, tussock-forming grasses of serious concern to the grazing industry across eastern Australia. GRT reduces the carrying capacity and productivity of more than 450,000 ha of pastoral land in eastern Queensland, New South Wales and areas of Victoria and is a high risk fodder contaminant. Current control efforts for weedy *Sporobolus* grasses centre on the use of chemical, mechanical, plant competition and pasture management. Despite these control options weedy *Sporobolus* grasses continue to rapidly spread into new areas.

Australian field surveys for culturable foliar pathogens of *Sporobolus* identified over 300 fungal isolates from at least 15 different genera. Eight of these genera (*Colletotrichum, Curvularia, Microdochium, Neopestalotiopsis, Pestalotiopsis, Phoma, Septoria* and *Stagonospora*) are known pathogens of grasses worldwide. Three of the isolates are recognised as new species and more isolates remain to be characterised. During the pathogen surveys, the South African GRT leaf smut, *Ustilago sporoboli-indici* has been found in the Queensland regional areas of Bundaberg, Childers, Conondale, Gin Gin, Miriam Vale and Taunton. The smut was found infecting a large number of GRT shoots indicating that it has the potential as a classical biocontrol agent in Australia.

The utilisation of endemic pathogens for controlling GRT in Australia at this stage remains untested. Pathogens of native Australian *Sporobolus* spp. may provide the arsenal, to control or suppress introduced weedy *Sporobolus* spp.

Keywords: *Sporobolus*, GRT, pathogen.

INTRODUCTION

Sporobolus (Poaceae) contains 186 grass species and potentially 12 unresolved species from tropical and subtropical parts of the world, including Africa, temperate and tropical Asia, Oceania, North and South America (Petersen *et al.* 2017). Eighteen species are endemic to Australia, and a further six species are naturalised weeds (Simon and Jacobs 1999; AVH 2019). The genus *Sporobolus* was divided into 15 major clades of closely allied species, including the *S. indicus* complex, which comprises at least 23 species (Petersen *et al.* 2017). In Australia, the *S. indicus* complex includes five introduced weedy species,

Parramatta Grass (*S. africanus*), Giant Parramatta Grass (*S. fertilis*), American Rat's Tail grass (*S. jacquemontii*), Giant Rat's Tail Grass (GRT) (*S. natalensis* and *S. pyramidalis*), as well as five native species, *S. blakei, S. creber, S. elongatus, S. laxus* and *S. sessilis*.

The weedy *Sporobolus* grasses threaten to cost the grazing industry of eastern Australia $60 million per annum, having the potential to infest 60% of Queensland and 30% of Australia over a range of soil types, where the annual rainfall is greater than 500 mm (Bray and Officer 2007). In Australian rangelands, *Sporobolus* species are undesirable pasture grasses that dominate pastures, excluding most other grass species (Burrows *et al*. 1988, Shrestha *et al*. 2003). The few native species regarded as favourable fodder grasses, *S. actinocladus, S. caroli, S. mitchellii* and *S. virginicus*, have high protein-content when fresh, but do not provide bulk (Simon and Jacobs 1999). Three of the native species, *S. disjunctus, S. latzii* and *S. partimpatens*, are considered rare (Simon 1993), and *S. pamelae* is listed as endangered in Schedule 2 of the Queensland Nature Conservation Act 1992.

In 2000, a biological control program was implemented by the Queensland Government and funded through Meat and Livestock Australia (Palmer 2008) to survey GRT and Parramatta grass in southern Africa for insects, mites and pathogens as potential biocontrol agents. The study identified 70 phytophagous insect species and 23 plant pathogens, with two agents, a wasp (*Tetramesa* sp.) and a leaf smut (*Ustilago sporoboli-indici*), showing promise (Palmer 2008). The wasp larvae feed in the stem of GRT resulting in malformation of the seed head (Palmer 2008). However all efforts to rear this species in the laboratory failed and work on this agent was discontinued in 2007 (Palmer 2008). The smut was found to infect four native Australian species of *Sporobolus* (all within the *S. indicus* complex section) (Yoko *et al.* 2009) and was therefore rejected as a biological control agent. In 2017, *Ustilago sporoboli-indici* was found infecting *S. natalensis* in Australia (Vitelli *et al* 2017), previously only known from South Africa on *S. pyramidalis*. The leaf smut produced black teliospores in sori in the leaves, leaf sheaths and stems which rendered infected shoots almost sterile (Vitelli *et al* 2017).

The potential of an Australian strain of the crown rot (*Nigrospora oryzae*) has been considered as a biological control agent for Giant Parramatta Grass *(S. fertilis)* (Ramasamy *et al.* 2008). When the crown rot was tested on GRT it was found to be ineffective (Fletcher and Leemon 2015).

Endemic plant pathogens are components of all natural ecosystems. For a disease to develop, three factors are needed: a virulent pathogen, a susceptible host, and a conducive environment. This project identified fungal pathogens of native *Sporobolus* spp at sites where both native and weedy *Sporobolus* species co-existed.

MATERIALS AND METHODS

Pathogen survey: Surveys for fungal pathogens were carried out across pastures, roadsides and forest verges of *Sporobolus* infested areas of Queensland, from Buchan Point (22 km N of Cairns) in the north to Beechmont (25 km SW of the Gold Coast) in the south during 2017-2019. These surveys were carried out every 30-60 days during the study period. The most extensive observations were made around Taunton (24.4587° S, 151.7992° E), near Miriam Vale, Queensland, where naturalised populations of GRT had been in decline since 2015.

At each location, five to 30 unhealthy plants exhibiting disease symptoms, including stunting, loss of vigour, inflorescence reduction or extensive lesions, were collected.

Priority was given to foliar diseases and root pathogens were excluded. Symptomatic plant material was removed and stored in labelled paper bags and infected tussocks were dug up and placed in labelled sterile resealable bags and placed in a cooler for transportation to the laboratories located at the Ecosciences Precinct (ESP), Dutton Park.

Pathogen isolation: For each infected plant, 3-5 cm segments of diseased leaf tissue were excised and surface sterilised by submersion in a solution of 10 % v/v ethanol and 1 % v/v sodium hypochlorite for 30 s. The leaf segment were then rinsed with sterile distilled water and dried on sterile filter paper inside a Laminar flow cabinet. Three to six 2 mm segments of leaf were then cut from the surface sterilised tissue adjacent to a lesion and placed onto a 9 cm plates with potato dextrose agar (PDA) supplemented with 0.05 % w/v streptomycin (SPDA) or 0.02% chloramphenicol (CPDA). The SPDA or CPDA plates were sealed with Parafilm®, labelled with a unique identifier, collection date and host plant, and placed in an airtight container and maintained in the dark in a growth cabinet set at 25 ↘C.

The plates were observed after 3 days for signs of fungal growth and checked periodically under a compound microscope (Leica DMLB). Fungal colonies were sub-cultured from the infected leaves. To prepare pure cultures, the hyphal tips were excised and sub-cultured onto PDA plates and incubated at 25↘C until pure cultures were observed. Mycelial plugs from pure cultures were inserted in 2 mL centrifugal tubes containing a 10% glycerol solution and placed in an -80↘C freezer maintained by the Queensland Plant Pathology Herbarium (BRIP) for long-term storage.

DNA – extraction, PCR and sequencing: Mycelium were scrapped off the PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (Qiagen). Genomic DNA was extracted with the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The primers V9G and ITS4 were used to amplify the internal transcribed spacer (ITS) region; primers T1 and Bt2b were used to amplify part of the β-tubulin gene (*tub2*); a partial region of the translation elongation factor 1-α (*tef1α*) locus was amplified using the primers EF1-728F and EF2, and the primers LR0R and LR5 were used to amplify partial regions of the large subunit (LSU) of the nuclear ribosomal RNA. All loci were amplified with Phusion High-Fidelity PCR Master Mix (New England Biolabs). The polymerase chain reaction (PCR) products were purified and sequenced by Macrogen Incorporated (Seoul, Korea).

RESULTS

Pathogen survey. 14 pathogen surveys were carried out across 64 *Sporobolus* infested sites in Queensland including Beechmont, Buchan Point, Bundaberg, Charters Towers, Clermont, Conondale, Dimbulah, Eton, Mackay, Mareeba, Miriam Vale, Mt Surprise, Taunton, Tewantin and Woodford.

A total of 159 tussocks and symptomatic plant material belonging to 13 species (*S. actinocladus, S. africanus, S. caroli, S. coromandelianus, S. creber, S. fertilis, S. jacquemontii, S. laxus, S. mitchelli, S. natalensis, S. pyramidalis, S. scabridus* and *S. virginicus*) were removed from the field, labelled and transported to ESP. From this material, 134 plants displayed foliar disease symptoms yielding over 300 fungal isolates.

Fast growing isolates that were easily identified morphologically as common saprobic fungi were not retained, e.g. *Penicillium* spp. Forty isolates were identified in 15 different genera

(Table 1) contain known plant pathogenic fungi. Eight of these genera (*Colletotrichum, Curvularia, Microdochium, Neopestalotiopsis, Pestalotiopsis, Phoma, Septoria* and *Stagonospora*) are known to contain fungal species pathogenic on grasses.

Three novel species of fungi (*Microdochium* sp. BRIP 65649, *Pestalotiopsis* sp. BRIP 66615 and *Neopestalotiopsis* sp. BRIP 66617) were found on hosts in the *Sporobolus indicus* complex. The novel identities of these isolates have been confirmed through phylogenetic analyses. Preliminary testing has shown that the three fungi play a role in GRT seedling mortality.

Table 1. Species details and BRIP accession numbers of isolates found on *Sporobolus* in eastern Queensland.

Host species	BRIP No.	Pathogen
S. fertilis	66084 a	*Alternaria arborescens*
S. natalensis	66616, 68299, 69018	[1]*Colletotrichum* spp.
Sporobolus sp.	68238/9, 68820	[1]*Colletotrichum* spp.
S. jacquemontii	66086 a, 66087 a, 66085 a	*Curvularia ravenelii*
S. natalensis	66088 a	*Curvularia ravenelii*
S. natalensis	69020	*Curvularia* sp.
S. natalensis	66081 a	*Exserohilum rostratum*
S. natalensis	65635 a	[1]*Fusarium sp.*
S. natalensis	66083 a	*Fusarium proliferatum*
S. natalensis	68300	*G*en. nov.
S. natalensis	68298	[1]*Microdochium* spp.
S. natalensis	65649, 67439a	[1,2]*Microdochium* sp. nov.
S. natalensis	66617	[1,2]*Neopestalotiopsis* sp. nov.
S. elongatis	68237	[1]*Neopestalotiopsis* sp.
S. natalensis		*Nigrospora* spp.
S. natalensis	66619	*Paraphaeosphaeria michotii*
S. natalensis	66615	[1,2]*Pestalotiopsis* sp. nov.
S. natalensis	65632 a, 63688 a, b, c	[1]*Phoma* sp.
S. natalensis	66618	[1]*Septoria* sp.
S. natalensis	65638 a	[1]*Stagonospora* sp.
S. natalensis	65466, 66039, 66324, 66325	*Ustilago sporoboli-indici*

[1]Species within this genus are known to be pathogenic grass fungi worldwide. [2]Three novel species found in this study to be pathogentic to *Sporobolus natalensis* (Lock 2018).

The pathogen surveys also uncovered further records of *Ustilago sporoboli-indici* in Australia (GRT leaf smut) on GRT. The systemic smut fungus produced sori in the leaves, leaf sheaths and stems rendering the infected plant shoot almost sterile. The leaf smut has been found in Queensland regional areas of Bundaberg, Conondale, Childers, Gin Gin, Miriam Vale and Taunton, spanning a distance of greater than 350 km.

DISCUSION

Once the exploration and isolation phase of the endemic *Sporobolus* study is complete (early 2020), isolates (currently 40) will be prioritised and Koch's postulates performed to confirm pathogenic effect and pathogenicity studies initiated. Pathogenicity tests will determine the potential of the fungi isolated during these surveys to affect the growth and development of weedy *Sporobolus* in Australia. Some of the fungi isolated from *Sporobolus* belonged to genera with species known to cause plant diseases of crops and pastures. For example, *Microdochium* includes species (*M. tainanense* and *M. trichocladiopsis*) pathogenic to sugarcane (*Saccharum officinarum*) and wheat (*Triticum aestivum*). If the fungi discovered during this study have potential as GRT inundative biological control agents, thorough host-range testing is essential to ensure that pasture species (native and introduced) and commercially important crops are not compromised.

Though not endemic the ability of *Ustilago sporoboli-indici* to infect a large number of shoots and render the plants almost sterile indicates that it has the greatest potential as a classical biocontrol agent in Australia. Pathogenicity testing will commence shortly. Investigations into the usefulness of *Ustilago sporoboli-indici* in an integrated control program will be dependent upon the availability of research funds.

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