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Biological control of weedy sporobolus grasses by two host specific agents

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Abstract

This report describes one of the first attempts at biological control of an invasive weedy grass. Five exotic *Sporobolus* spp., collectively known as the weedy sporobolus grasses, are serious weeds along the eastern seaboard of Australia. They are of extremely low palatability and cattle can not utilise them, and are also invasive and easily spread to new properties and areas. Biological control investigations commenced in 2000 with surveys of southern Africa, where *S. pyramidalis, S. natalensis* and *S. africanus* originate. Some 70 phytophagous insect species and 23 plant pathogens were found but only two organisms were considered potential biocontrol agents; the leaf smut *Ustilago sporoboli-indici* L. Ling and the stem wasp *Tetramesa* sp. These two agents were studied in this project. Techniques to culture the smut were developed and it was found to be infective for Australian populations of four of the target species, but not the American *S. jacquemontii.* However it was also infective on four Australian native *Sporobolus* spp. and was therefore rejected. All attempts to rear the stem wasp failed and as this is an essential prerequisite for further study, work on this agent was discontinued. Although other areas such as Asia and North America could be searched, the prospects for biological control of these grasses are not good.

Executive Summary

Five grasses (*Sporobolus africanus, S. fertilis, S. jacquemontii, S. natalensis,* and *S. pyramidalis*), collectively known as the weedy sporobolus grasses, are serious pastoral weeds in Australia affecting productivity, property management and ultimately land values. Because chemical and physical control methods are very expensive, biological control potentially offered a more practical solution. The Department of Natural Resources and Mines, Queensland, supported by Meat and Livestock Australia, undertook a project to find potential biocontrol agents. The search was conducted in southern Africa, through the Department's South African Field Station, because three of the grasses (*Sporobolus africanus, S. natalensis,* and *S. pyramidalis*) are native to that region.

An arthropod fauna of at least 70 species was found on the three weedy sporobolus grasses. The only insect seen as a prospective biological control agent was the eurytomid wasp, *Tetramesa* sp. The larvae of this wasp fed in the culm, which resulted in the malformation of the inflorescence and hence significant damage. The wasp was found at many localities throughout the survey area and often at high levels of infestation. Twenty-three pathogens, including five primary pathogens, were also found on the *Sporobolus* spp. Only the leaf smut *Ustilago sporoboli-indici* was thought to be a potential biological agent for Australia.

In this project, which followed on from the southern Africa survey for biocontrol agents, the objective was to develop the two potential agents, previously identified, through the various stages of research necessary before they could be approved for release in Australia for the biological control of the weedy sporobolus grasses. The plan also demanded that the weedy sporobolus grasses be approved as targets for biological control. If research results indicated host specificity, proposals for release in Australia would be written and submitted to Biosecurity Australia and the Department of Environment, Water, Heritage and the Arts for approval under the *Plant Quarantine Act 1908* and the *Environment Protection and Biodiversity Conservation Act 1999*.

Application was made through the Australian Weeds Committee for all five species of weedy sporobolus grasses to be approved as targets for biological control. This process is undertaken for all weeds being considered for biological control to ensure that that the weed does not have any significant utility to any sector of society ('one man's weed may be another man's crop'). In this case, there was anecdotal evidence that some graziers considered *Sporobolus fertilis* to be a useful drought reserve. However, approval was obtained in August 2007 from the Natural Resource Management Standing Committee for all five species to be targets for biological control. This step is necessary before approval can be obtained for the release of agents.

The leaf smut *Ustilago sporoboli-indici* was considered the better prospect. Because suitable quarantine facilities to house a plant pathogen were not available to the project in Australia, it was decided that all research on this organism would be conducted in South Africa at the University of KwaZulu-Natal by Dr. Kwasi Yobo under the supervision of Professor Mark Laing. The research considered necessary included biology studies, development of infection and culturing techniques, determining that the leaf smut was infective for Australian populations of the five weedy species, and determining that leaf smut would not attack any Australian native or forage grasses.

Early in the project it was found that basidiospores or inoculum obtained from washed agar plates dusted with teliospores and germinated overnight was more effective and quicker to cause symptoms of infection on susceptible *Sporobolus* grasses than when inoculum was generated from continuous or submerged broth culture.

Seeds of all five weedy sporobolus grasses were collected in Australia and sent to South Africa where they were germinated for testing. Tests showed that *Sporobolus pyramidalis*, *S. africanus*, *S. fertilis* and *S. natalensis* were susceptible and hence hosts for the smut fungus, *Ustilago sporoboli-indici*. However the fifth species, *S. jacquemontii,* a native of America, did not show any typical symptoms of infection even after prolonged periods of inoculation (90 day post-inoculation) and it was concluded that this species was not a host.

Host range trials with the smut fungus against 10 native Australian *Sporobolus* species indicated that four species (*S. creber, S. elongatus, S. sessilis* and *S. scabridus*) developed symptoms of infection and that *S. creber* and *S. elongatus* were seriously infected. A further evaluation of extent of damage caused by the smut to two weedy (*S. fertilis* and *S. natalensis*) and two native Australian (*S. creber* and *S. elongatus*) *Sporobolus* species showed that there were no significant differences between each of the inoculated four grass species and their respective uninoculated controls in terms of numbers of tillers with flowers formed and dry biomass and that *S. creber* had the highest percentage of infected tillers/flowers followed by *S. fertilis*, *S. elongatus* and *S. natalensis* in that order.

The results of the testing of the leaf smut were sent to a group of Australian experts for their comment, particularly as to whether the leaf smut justified further research to see whether a case justifying release in Australia could be developed. The group included biosecurity administrators, plant pathologists, botanists, pasture agronomists biocontrol practitioners and weed research leaders. There was little enthusiasm for further research on the leaf smut and work was terminated.

The second organism of interest was the stem wasp *Tetramesa* sp. The larvae of this wasp feed in the culm resulting in the malformation of the inflorescence and hence significant damage. The critical issue for the wasp was whether it could be successfully reared in the laboratory as this was an essential prerequisite before it could be shipped to Australia for host range studies in quarantine. A one year study was therefore undertaken in 2006 at the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI) centre at Rietondale to investigate its biology and to develop a method of laboratory culture. All efforts to rear *Tetramesa* sp. in the laboratory were unsuccessful. Supplementary biology studies suggested that the wasp might have a winter diapause mechanism and that its effect on the plant may be less than originally thought. Further work on the insect was therefore also terminated.

This project represents one of the first attempts to manage a weedy grass by biological control. Grasses present a formidable target in that they are economically and ecologically so valuable, they are adapted to significant predation by animals, and they may have fewer host specific arthropod and pathogen associates. This particular project was more difficult because there were so many native Australian *Sporobolus* spp. resulting in a really very high degree of host specificity being demanded. The result remains very disappointing because the weedy sporobolus grasses continue to become more significant weeds with few satisfactory management methods available to land managers.

Contents

1 Background

1.1 The weedy sporobolus grasses

Five grasses (*Sporobolus africanus, S. fertilis, S. jacquemontii, S. natalensis,* and *S. pyramidalis*), collectively known as the weedy sporobolus grasses, are serious pastoral weeds in Australia affecting productivity, property management and ultimately land values (Bray and Officer 2007). The detrimental effects of these grasses are such that the potential annual losses to beef production in northern Australia, if weed sporobolus grasses spread to their limits, have been estimated at $60 million/year (Walton 2001).

The weedy sporobolus grasses are all exotic and belong to a section of the *Sporobolus* genus known as the *indicus* complex (Simon and Jacobs 1999). The species included in the *indicus* complex are morphologically very similar and it is quite likely that these species will be redefined should appropriate molecular studies be conducted. The *indicus* complex is therefore presently represented in Australia by 11 species including 6 native species. A further 13 species outside this complex complete the 24 *Sporobolus* spp. found in Australia.

Three of the weedy species (*Sporobolus africanus, S. natalensis,* and *S. pyramidalis*) originate in southern Africa (Simon and Jacobs 1999). *Sporobolus fertilis* is of Asian origin while *S. jacquemontii* is of American origin, Southern Africa was therefore a logical starting point for a search for biological control agents. Further, it was also logical to conduct the search from an existing biological control facility, the Queensland Department of Natural Resources & Mines' South African Field Station (Anon 2002).

1.2 Biological Control

1.2.1 General

Biological control offers a cost effective method of reducing the detrimental economic effects of this weed complex in the longer term. Biological control seeks to alter the presently favourable dynamics for the weed thereby weakening the weed's ability to compete with other plant species in the sward (McFadyen 1998). A typically successful biocontrol might return a benefit/cost ratio of $2-10 per research dollar and in some cases this is considerably higher (Page and Lacey 2006).

A typical classical biological control project involves ascertaining the origin of the weed, surveying for natural enemies in its land of origin, testing prospective agents to ascertain they are safe to release in Australia, mass rearing and releasing the agent if approved for introduction, and then evaluating the effect of the agent after it has established (Harley and Forno 1992, Julien and White 1997).

1.2.2 Biological Control of Grasses

Weedy grasses have only recently been targeted for biological control (Witt and McConnachie 2004). They have not been considered good targets for a number of reasons including the great economic and ecological importance of related species, the simple chemical composition and morphology of grasses which may preclude any great degree of speciation, and the great adaptability of grasses to grazing and harvesting (Palmer *et al*. 2008).

1.3 Previous work

A study of potential biological agents in southern Africa for the weedy sporobolus grasses was undertaken over a two year period, 2001-2003, from the South Africa Field Station situated near Pretoria, South Africa (Palmer 2004, Witt and McConnachie 2004).

The survey was primarily undertaken by the full time staff of the South African Field Station; Arne Witt and Andrew McConnachie; both entomologists by training. They were joined on some trips by Dr Isabel Rong who identified the pathogens. In December 2002 Dr Roger Shivas and Dr Kalman Vánky, a smut specialist, spent a month in South Africa searching for further pathogens.

Sporobolus pyramidalis, S. natalensis and *S. africanus* are native to southern Africa and the study involved surveying the phytophagous arthropod fauna and pathogens on all three grasses throughout as much of their range as possible. In that respect it was not possible to visit some countries, such as Zimbabwe, because of political and safety issue. Ultimately, South Africa, Botswana and Swaziland were surveyed. A second difficulty was that southern Africa experienced drought conditions similar to Australia for much of the two years of the project.

Identification of the individual species of *Sporobolus* was difficult as they are morphologically quite similar, they interbreed and the taxonomy is problematic. *Sporobolus pyramidalis* and *S. natalensis* did not occur in the Western Cape whereas *S. africanus* was quite abundant in pastures in this region. All three species co-occur in areas further north and are particularly abundant in disturbed sites.

An arthropod fauna of at least 70 species was found on the three weedy sporobolus grasses. Most of these species represented casual associations with the plant rather than utilizing the grass as a host plant. Many of the species were only partially determined (usually to genus) as they belonged to groups that have not yet been properly described in southern Africa.

The only insect seen as a prospective biological control agent was the eurytomid wasp, *Tetramesa* sp. The larvae of this wasp feed in the culm which results in the malformation of the inflorescence and hence significant damage. The wasp was found at many localities throughout the survey area and often at high levels of infestation. Attempts to rear this insect in the laboratory were unsuccessful. Up to four other undescribed eurytomid wasp species, some possibly parasitic, were also be found in the stems.

Twenty three pathogens, including five primary pathogens, were found on the *Sporobolus* spp. At the conclusion of the December 2002 survey by Dr Shivas and Dr Vánky, it was concluded that only the smut *Ustilago sporoboli-indici* — described by Vanky (2003) — was a potential biological agent for Australia. On his return to Europe, Dr Vánky conducted follow up studies and was successful in germinating spores of *U. sporoboli-indici* in his laboratory.

2 Project Objectives

The project had the following objectives to progress the possibility of the weedy sporobolus grasses being brought under biological control in Australia:

(1) To have all five weedy sporobolus grasses approved as agents for biological control.
(2) To conduct all necessary research at the University of KwaZulu-Natal, South Africa on the biology, culturing techniques and host range of the leaf smut, *Ustilago sporoboli-indici* such that its release in Australia could be considered.
(3) To conduct all necessary research at the ARC-Plant Protection Research Institute, South Africa, and the Alan Fletcher Research Station, Brisbane on the biology, rearing techniques and host range of the stem wasp, Ustilago sporoboli-indici such that its release in Australia could be considered.
(4) If appropriate, to prepare proposals for the release of the above agents for consideration by the relevant Australian Government entities and to champion any proposals through the

steps towards approval. A successful outcome at the end of the project would be approval of both agents for release in Australia.

3 Methodology

3.1 Approval as targets for biological control

A very early step in any biological control project is to have the target weed approved by Natural Resource Management Standing Committee. This is done by submitting a proposal to the Australian Weeds Committee which then canvasses the opinion of relevant departments from all states and territories. The purpose of this process is to determine whether there are any conflicts of interest within the Australian community and whether there is general agreement the weed should be controlled biologically. Once biological control agents are introduced their effects are generally irreversible and often difficult to contain.

The proposal would review known information about the weed including its taxonomy, phylogeny, biology, origin, Australian distribution and abundance. Closely related plants of economic or ecological importance are identified. The extent and significance of weed problem and alternate methods of control are also identified. It is critically important to identify any perceived beneficial attributes of the weed.

3.2 Investigations into the leaf smut

The leaf smut *Ustilago sporoboli–indici* was studied at the University of KwaZulu-Natal from 2005 to 2006. The first step was to develop a satisfactory laboratory method of culture and a knowledge of the life cycle of the smut. The pathogenicity of the smut fungus was tested against Australian populations of the five weedy sporobolus grasses (*S. pyramidalis, S. africanus, S. fertilis, S. natalensis* and *S. jacquemontii*).

A primary screen for host range was then undertaken. This involved testing the smut against 10 species of *Sporobolus* native to Australia. These were: *S. actinocladus, S. contiguous, S. coromandelianus, S. creber, S. disjunctus, S. laxus, S. scabridus* and *S. sessilis*. If the smut were to cause serious damage to any of these species, it would most likely lead to rejection of the agent. Details of the methods used for all these studies are provided in Appendix 2.

3.3 Investigations into the stem wasp

The stem wasp *Tetramesa* sp. was studied for a year at the Rietondale laboratory of the ARC-PPRI. The primary objective of this study was to develop a method whereby this insect could be cultured satisfactorily in the laboratory. This being an essential step before it could be shipped to an Australian quarantine for host specificity studies. In the course of this investigation it was hoped to gain useful information on the insect's distribution and abundance, its biology, and its ability to damage the plants under laboratory conditions. Details of the methods used for these studies are given in Appendix 2.

3.4 Proposals to release agents

Biological control agents are not released in Australia unless they are approved by both AQIS and the Department of Environment, Water, Heritage and the Arts for approval under the *Plant Quarantine Act 1908* and the *Environment Protection and Biodiversity Conservation Act 1999* respectively. An essential precursor to applying for release is that the target weed is already approved for biological control.

Assessment on the suitability of an agent organism is based on a risk assessment as to whether they might cause detriment to other economic and native organisms. This assessment is made primarily on the agents host specificity although the effectiveness of the agent can also be a consideration. A case must therefore be put that the agent is sufficiently host specific and that it poses little risk to Australian crops, pastures and the environment.

Submissions would be written proposing the release of *Ustilago sporoboli-indici* and *Tetramesa* sp. if the scientific data assembled at the University of KwaZulu-Natal, the ARC-PPRI and within the quarantine at Alan Fletcher Research Station justified this progression. Submissions would be submitted to both Biosecurity Australia (who make recommendations to AQIS) and to the Department of Environment, Water, Heritage and the Arts and defended should adverse comment be forthcoming from their reviewers.

4 Results and Discussion

4.1 Approval as targets for biological control

A proposal (Appendix 1) to have all five weedy sporobolus species approved as targets for biological control was submitted to Australian Weed Committee. One particular potential issue was the anecdotal information that some graziers, particularly in the Northern Rivers region of NSW regarded Giant Parramatta grass. *Sporobolus fertilis*, as a useful drought reserve. However this and the other species, were declared noxious weeds in both Queensland and NSW.

The weedy sporobolus grasses were approved as targets for biological control in August 2007.

4.2 Investigations into the leaf smut

Details of the results of the leaf smut investigations are given in full in Appendix 2.

4.2.1 Pathogenicity of weedy sporobolus grasses

Basidiospore suspension inoculated onto weedy sporobolus grass seedlings successfully caused infections typical of *U. sporoboli-indici* on four of the five weedy *Sporobolus* spp. found in Australia. *Sporobolus pyramidalis*, *S. africanus*, *S. natalensis* and *S. fertilis* were all infected by the smut fungus but not *S. jacquemontii*. Infections were seen on seedlings of all four susceptible species as early as 4-6 weeks after inoculations. No symptoms were seen on *S. jacquemontii* even after 90 days after inoculation. Sori on leaves and, in some cases, on stems were seen on infected plants. None of the seedlings treated with basidiospores of *U. sporoboli-indici* died by the end of the experiment. Basidiospores generated from teliospores collected from *S. pyramidalis* caused infections on all four susceptible *Sporobolus* spp. and *vice-versa* for teliospores collected from *S. africanus*.

4.2.2 Pathogenicity of native Australian *Sporobolus* spp.

Of the 13 native Australian species tested, four were attacked by *U. sporoboli-indici*. These were *S. creber*, *S. elongatus*, *S. sessilis* and *S. scabridus*. *Sporobolus creber* and *S. elongatus* were seriously attacked by the smut fungus, while *S. sessilis* and *S. scabridus* developed minor infections. Severe infections on *S. creber* and *S. elongatus* resulted in dead leaves, flower malformations with production of teliospores in leaves and tillers. Plants very severely attacked by the smut fungus resulted in the absence of inflorescences or production of sterile inflorescences.

4.2.3 Electron microscopy studies

Teliospores of *U. sporoboli-indici* successfully germinated and penetrated sporobolus grasses after being dusted onto the plants. Mycelial network were seen on leaf surfaces following germination of teliospores. Penetrations of the smut through the stomata guard cells and through the epidermis were also seen on samples after 72 h. Similar results were observed on all three grasses examined.

4.2.4 Effect of leaf smut

Sporobolus creber had the highest number of flowers formed and the highest number of infected flowers; with 21.07% of total flowers formed becoming infected. The total numbers of flowers infected were in the following order: *S. creber* (21.07%)> *S. fertilis* (14.17%) > *S. elongatus* (12.09%) > *S. natalensis* (2.80%) and significant differences were found when the numbers of infected flowers on each treatment were compared (P = <0.001). No significant difference was found for dry biomass when each of the treated grass species was compared with their respective untreated controls. Only *S. fertilis* recorded a significantly different comparison (P = 0.04) when biomass of treated group was compared with its untreated control group for the mean number of flowers formed.

4.3 Investigations into the stem wasp

Details of the results of the stem wasp investigations are given in full in Appendix 3.

The stem wasp was found to be quite abundant at several sites within a day's drive of Pretoria and it was not difficult to collect material for laboratory studies.

The stem wasp was found in the field throughout the year. Larvae generally were more abundant in autumn and winter while pupae were found more often in culms in summer. The wasp was considered to be multivoltine with several generations per year. A larval diapause was suspected.

All attempts to culture the insect in the laboratory were unsuccessful. Very few wasps emerged from infested grass stalks brought back from the field. Oviposition was not observed and there was no evidence of damage or immature development in uninfested plants offered to the emerging adults.

4.4 Proposals to release agents

Neither prospective agent was successfully advanced to the stage when an application for release in Australia could be considered. The opinions obtained from various stakeholders in relation to the leaf smut are given in Appendix 4.

5 Success in Achieving Objectives

The project was partially successful.

The weedy sporobolus grasses were all approved as targets for biological control by Natural Resource Management Standing Committee. That approval is not time-limited meaning that future efforts in biological control need not reapply for approval. In the process of application the weeds, collectively, have been brought to the attention of weed policy makers in all states and the Australian Government and relevant literature and research summarised.

Although the leaf smut did not prove to be sufficiently host specific for release in Australia, much was learned about it. In the process studies in these projects have contributed to a better understanding of the taxonomy of the genus *Ustilago.*

Very little was achieved with the stem wasp. All efforts to breed the insect in captivity were unsuccessful. As a consequence we were not able ship the insect to Australia and commence host specificity studies within quarantine.

6 Impact on Meat and Livestock Industry

The weedy sporobolus grasses remain a very serious threat to the cattle industries and a solution by biological control would have made a very valuable contribution. These grasses are relatively recent introductions to Australia and most likely have not reached their ultimate distribution nor their maximum densities.

7 Conclusions and Recommendations

Southern Africa, the native range for three of the five weedy sporobolus grasses was a logical starting point for investigations to find suitable biological control agents. Although there were constraints limiting the exploration of the area caused by the prevailing political situation, eventually a very satisfactory survey produced only two possibilities for further investigation. Unfortunately neither of these have proved to be suitable for implementation as biocontrol agents.

Quite recently, a pathogen already present in Australia has come to the attention of those working on the weedy sporobolus grasses. This pathogen, a fungus, *Nigrospora oryzae*, was seen to be adversely affecting *S. fertilis* in the Grafton area by Mr David Officer. Preliminary studies conducted by Dr Ann Lawrie and colleagues at the RMIT University have demonstrated its pathogenic effect on this grass and indicated that there may be potential for the development of a mycoherbicide based on this pathogen. The effectiveness of the fungus against the other four weedy sporobolus grasses has not been determined. The preliminary investigations on this fungus indicate that further research on it will be justified.

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9 Appendices

9.1 Appendix 1: Nomination of weeds as biocontrol targets

NOMINATION OF TARGET WEEDS
FOR
BIOLOGICAL CONTROL

Targets: **Weedy Sporobolus Grasses**
Giant rats tail grasses
Giant Parramatta grass
Parramatta grass
American rats tail grass

Nominating Organisations:

Queensland Department of Natural Resources and Mines
NSW Department of Agriculture
Victorian Department of Primary Industries

1. Taxonomy

Order: Cyperales
Family: Poaceae
Subfamily: Chloridoideae
Tribe: Cynodonteae
Genus: *Sporobolus*
Section: *Sporobolus* (or *S. indicus* complex)
Species:

(1) *Sporobolus pyramidalis* P. Beauv. 1816
Common name: Giant rats tail grass

(2) *Sporobolus natalensis* (Steud.) Dur. & Schinz. 1865
Common name: Giant rats tail grass

(3) *Sporobolus africanus* (Poir.) Robyns & Tournay 1955
Common name: Parramatta grass

(4) *Sporobolus fertilis* (Steud.) Clayton 1965
Common name: Giant Parramatta grass

(5) *Sporobolus jacquemontii* Kunth 1831
Common name: American rats tail grass

Although the genus Sporobolus has been revised recently (Simon and Jacobs 1999), the taxonomy of these particular species is not completely clear. All are morphologically similar with identifying traits often overlapping. Genetic studies have also indicated great similarity and have supported the concept of treating the five species as a group, the weedy sporobolus grasses.

2. Native Range and Centre of Origin

The Poaceae is one of the largest and most cosmopolitan of the flowering plant families of the woorld with more than 700 genera and about 10,000 species. Twelve subfamilies and more than 40 tribes are recognised. The Poaceae provides the world's three major grain crops and the basis of the diet of many domestic livestock and wild herbivopre species (McCusker 2002). The Poaceae possibly originated in Africa or South America (Macphail and Hill 2002).

The subfamily Chloridoideae is the least well known phylogenetically of the twelve subfamilies. While morphological evidence is often weak, molecular data consistantly support monophyly. All species but one are C4 (Kellogg 2002).

The genus *Sporobolus* consists of about 160 species in tropical and subtropical areas of the world and *Sporobolus* spp. are found in mesophytic, xerophytic and halophytic regimes. They grow in diverse habitats and there are both facultative annual and perennial species. The world wide distribution of the genus is reflected in its presence in almost all but the polar floristic regions. However there is a high level of endemism in Africa, Australasia and both North and South America (Simon and Jacobs 1999).

Attempts to divide the genus into a number of well defined sections have been largely unsuccessful but clusters of closely allied species have been recognised. One such cluster is the world wide weedy *S. indicus* complex. This complex has elements originating in both the New World and Africa.

3. Australian and overseas distribution

Sporobolus pyramidalis is of African origin where it occurs throughout tropical Africa as well as on Mauritius, Madagascar and Yemen (van Oudtshoorn 1999). In Australia, it is found principally in coastal areas from Mareeba in northern Queensland to the northern rivers of NSW. However its potential distribution includes coastal areas from Broome in the north-west to Sydney in the south.

Sporobolus natalensis is also of African origin where it occurs particularly in central and southern Africa. In Australia, it is found principally in coastal areas from Rockhampton, Queensland to the northern rivers of NSW.

Sporobolus africanus is of African origin where it occurs from southern Africa to to east Africa as far north as Ethiopia (van Oudtshoorn 1999). However it appears to have a more temperate distribution than *S. pyramidalis* as it is found in both the Eastern Cape and Western Cape provinces of South Africa. In Australia it is now widespread on coastal soils in northern NSW and in northern Vicoria and Gippsland. Its potential distribution includes relatively coastal areas of eastern Australia from Cape York to Adelaide and also the south-west corner of Western Australia.

Sporobolus fertilis is thought to be native to tropical Asia and Malesia. It one time it was thought to be native to Australia. Presently in Australia, very heavy infestations occur in northern New South Wales and it is spreading south and north along the coasts. . However its potential distribution includes coastal areas from Cape York in the north through to the South Australian coast in the south and also south-western Western Australia.

Sporobolus jacquemontii is of tropical American origin but has been established in Australia for a long time. Its current distribution in Australia is primarily in Queensland, particularly in the Mackay/Proserpine and lower Burdekin regions, but also in the Northern Territory.

4. Native and introduced related species

The subfamilies, tribes and genera of Poaceae represented in Australia are given by Kellogg (2002).

The subfamily Chloridoidae is represented in Australia by three tribes and 36 genera (table 1). The subfamily includes a number of important genera such as *Astrebla, Chloris* and *Brachyachne.*

While there are about 160 *Sporobolus* spp. world wide, 24 taxa are currently recognised in Australia (Simon and Jacobs 1999). Fourteen of these are endemic or native to Australia. Although none of these has a rare or threatened status three species, *S. pamelae, S. partimpatens* and *S. disjunctus*, are under consideration for listing under the Queensland Nature Conservation Act.

There are eleven species in the *S. indicus* complex found in Australia. Five of these are covered in this submission. Six species (*S. blakei, S. creber, S. disjunctus, S. elongatus, S. laxus* and *S. sessilis*) are considered native species. *Sporobolus blakei* occurs in arid regions. *Sporobolus creber, S. elongatus, S. laxus* and *S. sessilis* grow along the eastern coast and would be sympatric with the weedy sporobolus grasses. *Sporobolus disjunctus* occurs on black, cracking clay soils from central Queensland to the Darling Downs and is under consideration for listing under the Queensland Nature Conservation Act as a rare species.

5. Proposing Organisation

Queensland Department of Natural Resources & Mines

6. Pest Status

The weedy sporobolus grasses are regarded as serious weeds of pasture and the environment. Their deleterious effects have been well documented and are summarised from the Weedy Sporobolus Grasses Strategy (Walton 2001)as follows:

Primary Production:

- Control costs to primary industry are high and operations difficult due to the nature of the species and adjacent pasture species, however, these costs need to be better documented,
- Reduce pasture production and hence reduction in carrying capacity. Farmers have stated losses in carrying capacity and production ranging from 10-80%, depending on density of infestations,
- Reduce animal production. Reports that cattle grazing pastures infested with Giant Parramatta grass took up to 12 months longer to reach equivalent weights to those grazing in uninfested pastures and losses of 20% are claimed by some farmers where weedy sporobolus have taken over. Based on these figures the annual loss to beef production in northern Australia could be in the vicinity of $60 million/year, if weed sporobolus grasses spread to their limits,
- In Victoria, farmers have reported dairy herd milk production dropping by 100's of litres when entering pasture dominated by Parramatta grass,
- The cost of producing milk on two giant Parramatta grass infested properties in the Rosedale/Miriam Vale area has increased by 15 and 25%,
- The tough fibrous nature of stems can increase teeth wear of stock resulting in reduction of productive life, and
- Reduction of land values in heavily infested areas.

Environmental:

- With the exception of Giant Parramatta grass, there are significant species replacements in swampy soils, which leads to degradation of these sensitive areas,
- May invade open native and plantation forests where it may not affect production but will impact on native species. These grasses do not grow well where there is heavy canopy cover and as a result forestry plantations are seen as control option for weedy sporobolus in non-arable or steeply sloping lands,
- Giant rats tail grass is of concern in eight conservation areas of Queensland; 1 in Far North region, 4 in North region and 3 in Central Coast regions. Impacts of others species and in other states have not been catalogued,
- Infestations result in grass monoculture, reducing biodiversity of ground cover species and potentially native herbivores which also find the plants unpalatable, and
- Heavy infestations may increase fire intensity in sensitive environmental areas.

Tourism and amenity:

- A problem in disturbed sites in natural areas, such as tracks and cleared areas resulting in increased management costs and decrease in aesthetic appeal,
- Infestations may spread into surrounding undisturbed areas in bad seasons,
- Infestations in these areas are a source of seed which can contaminate clean areas,
- Cause damage to asphalt on roadsides and tracks, and
- Large infestations may affect fire intensity or frequency.

All five weedy sporobolus grasses are declared as Class 2 pest plants under the Queensland Land Protection (Pest and Stock Route Management) Act 2002. In NSW, both *S. fertilis* and *S. pyramidalis* are declared as either Category W2 or W3 weeds, depending upon region.

7. Other Methods of Control Available

The weedy sporobolus grasses are not presently under effective and economical control. Different approaches and control methods are being utilised for managing weedy sporobolus grasses in particular climatic regions and land uses. These controls depend on the infestation density, existing pasture species, type and level of agricultural production, and type of land and soil. Timing is also very important for actions, to focus on control before plant maturity.

Grazing management, chemical methods, and possibly fire make up an integrated control program for weedy sporobolus. Mechanical control is generally inappropriate except for occasional plants. Buffer strips between infested and non-infested areas are very effective but require regular follow-up. Slashing has been a traditional method of seeding management, but this method will cause the spread of these weeds. In addition to this, slashing frequency, labour costs and impacts on desirable pasture species greatly diminish the suitability of this method of weed control. Wick herbicide application provides effective treatment of taller grass species leading to positive changes in botanical composition of pastures. Success of this method of control depends on accurate timing and requires slashing before applications. Flupropanate is a relatively expensive selective herbicide that allows weedy sporobolus to be removed from some mixed pasture swards. The commonly used herbicide glyphosate requires actively growing plants and is not selective.

Due to the widespread distribution of weedy sporobolus grasses and difficulty to control within pasture situations, grazing management to minimise impacts on pasture production, whilst improving utilisation of plants, appears to be the best approach for a management strategy. In Zimbabwe, native grasses, including Rhodes grass, are used to ensure good grass cover to minimise sporobolus growth. Maintaining continuous grazing (set stocking) increases the level of weedy sporobolus grasses in pastures, whilst removal of stock results in an increase in more palatable grasses. Dense stands of desirable pasture species may prevent establishment of weedy sporobolus, however this may not be possible in regions with unreliable rainfall. Young sporobolus leaf has some grazing value. Work in New South Wales shows that wick wiping in summer at low glyphosate rates can improve production of green pick while reducing seed production. Research is continuing on appropriate feed management, including grass value and competitive pasture species. When cultivation is possible, grain or oilseed cash crops and pasture replanting have been used. Controls have not been well developed for undisturbed sites or native pastures, as economics of control in low carrying capacity areas are not currently cost effective.

8. Potential Conflicts of Interests

The weedy sporobolus grasses are universally regarded as weeds although there is anecdotal evidence that some landholders in NSW may regard *S. fertilis* as a useful drought reserve.

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Table 1: Tribes and genera of the subfamily Chloridoidae having endemic or naturalized species in Australia (Kellogg 2002), together with some representative significant species (Lazarides 2002).

Tribe	Genera in Australia	Significant species
Pappophoreae	*Enneapogon*	*E. avenaceus, E. caerulescens, E. nigricans*
Triodieae	*Monodia*	
	Symplectrodia	
	Triodia	*T. basedowii, T. irritans, T. pungens, T. schinzii*
Cynodonteae	*Acrachne*	
	Astrebla	*A. elymoides, A. pectinata*
	Austrochloris	
	Brachyachne	*B. convergens*
	Chloris	*C. gayana, C. truncata*
	Crypsis	
	X Cynochloris	
	Cynodon	*C. dactylon*
	Dactyloctenium	*D. radulans*
	Dinebra	
	Distichlis	*D. distichophylla*
	Ectrosia	
	Eleusine	
	Enteropogon	*E. acicularis*
	Eragrostiella	
	Eragrostis	*E. falcate, E. lanipes, E. xerophila*
	Eustachys	*E. distichophylla*
	Heterachne	
	Leptochloa	*L. decipiens,*
	Lepturus	*L. repens*
	Microchloa	
	Oxychloris	
	Perotis	*P. rara*
	Planichloa	
	Psammagrostis	
	Spartina	
	Sporobolus	*S. actinocladus, S. australasicus, S. caroli, S. elongatus*
	Thellungia	
	Tragus	*T. australianus*
	Tripogon	
	Triraphis	
	Zoysia	*Z. macrantha*

9.2 Appendix 2: Report on leaf smut by UKZN, South Africa

PROJECT REPORT

Classical Biological Control of Weedy Sporobolus Grasses by the Smut Fungus *Ustilago sporoboli-indici*

Phases 1 and 2

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Executive Summary

- For the past two years (2005-2006), we have been evaluating the potential of the smut fungus, *Ustilago sporoboli-indici* for classical biological control of five weedy *Sporobolus* grasses in Australia.
- From all indications, basidiospores or inoculum obtained from washed agar plates dusted with teliospores and germinated overnight was more effective and quicker to cause symptoms of infection on susceptible *Sporobolus* grasses than when inoculum was generated from continuous or submerged broth culture.
- The results obtained from the pathogenicity trials against the five weedy alien invasive *Sporobolus* grasses indicate that four (4) viz: *Sporobolus pyramidalis*, *S. africanus*, *S. fertilis* and *S. natalensis* out of the five grasses were susceptible and hence a host for the smut fungus, *Ustilago sporoboli-indici*.
- Only *S. jacquemontii* did not show any signs or symptoms of infection typical of the smut fungus. *S. jacquemontii* still showed no symptoms of infection after prolonged periods of inoculation with the smut fungus (90 day post-inoculation).
- Absence of infection in *S. jacquemontii* after 90 days post-inoculation period with the smut indicates that *S. jacquemontii* is probably a non-host to the smut fungus.
- Young seedlings responded quicker to infection than older seedlings, irrespective of the type of inoculum used. Teliospores collected from *S. pyramidalis* caused infection in all four susceptible weedy grass species and *vice versa* for teliospores collected from *S. africanus*.
- Host range trials with the smut fungus against 10 native Australian *Sporobolus* grass species indicated that four (4) species: *S. creber*, *S. elongatus*, *S. sessilis* and *S. scabridus*, out of the 10 native Australian *Sporobolus* grass species, developed symptoms of infection typical of the smut fungus. Among these four native *Sporobolus* grass species, *S. creber* and *S. elongatus* were seriously infected. Infections on the two other species: *S. sessilis* and *S. scabridus*, were minimal and did not spread to other uninfected leaves of the same plant. Hence, infections remained localised.
- Evaluation of extent of damage caused by the smut to two weedy (*S. fertilis* and *S. natalensis*) and two native Australian (*S. creber* and *S. elongatus*) *Sporobolus* species showed that there were no significant differences between each of the inoculated four grass species and their respective uninoculated controls in terms of numbers of tillers with flowers formed and dry biomass.
- Comparison of all four inoculated grass species with the smut without their uninoculated controls showed that *S. creber* had the highest percentage of infected tillers/flowers, followed by *S. fertilis*, *S. elongatus* and *S. natalensis* in that order.
- It is worth noting that the degree of infections was not uniform in seedlings of all pots, and within the same grass species. Some seedlings gets infected and damaged more while infections and damages in some other plants are minimal.
- Generally, the development of symptoms of infection on all susceptible grass species, whether native or alien, is slow. However, symptoms on *S. creber* and *S. elongatus* develop much quicker and faster than infections on *S. pyramidalis*, *S. africanus*, *S. fertilis* and *S. natalensis*. However, we are not intimating that *S. creber* and *S. elongatus* are better hosts than the four susceptible weedy species listed above.
- Should this fungus be considered as a possible classical biological control agent for the alien invasive weedy *Sporobolus* grass species, methods that enhances its infection on the alien *Sporobolus* grass species should be encouraged and developed.

1. Project Objectives

Five *Sporobolus* species: *S. pyramidalis*, *S. africanus*, *S. fertilis*, *S. natalensis* and *S. jacquemontii,* are weedy invaders of Australian grasslands. A smut fungus, *Ustilago sporoboli-indici*, has been found on *Sporobolus* spp. in South Africa. This smut attacks flowering parts of the *Sporobolus* spp. found in South Africa and has been earmarked as a potential biological control agent for the five weedy *Sporobolus* invaders found in Australian grasslands.

The main purpose of this project is to develop and assess the potential of *Ustilago sporoboli-indici* for classical biological control of the five weedy *Sporobolus* species found in Australia. The project has been divided into two phases. Phase 1 of the project carried out the following main objectives:

Phase 1

1. To develop a satisfactory laboratory method of culture and a knowledge of the life cycle of the smut fungus, *Ustilago sporoboli-indici*.
2. To test the pathogenicity of the smut fungus against Australian populations of the five African species of weedy *Sporobolus* grasses (*Sporobolus pyramidalis*, *S. africanus*, *S. fertilis*, *S. natalensis* and *S. jacquemontii*).

Phase 2

3. To conduct a primary screen for a host range involving testing the smut against 10 species of *Sporobolus* native to Australia. These are: *S. australasicus*, *S. coromandelianus*, *S. laxus*, *S. disjunctus*, *S. scabridus*, *S. contiguus*, *S. creber*, *S. sessilis*, *S. actinocladus* and *S. caroli*. If the smut were to cause serious damage to one of these species, this would stop the project.

Phase 3

4. Test *Ustilago sporoboli-indici* against a range of Australian flora. If all three phases successful, then proceed to release in Australia.

2. Progress during 2005

2.1 Meetings

Project meetings were held once every month (3rd or 4th week) with Professor Mark Laing. The main objective of the meetings was to discuss the progress of the project. Matters/issues relating to the progress, future works, as well as difficulties encountered during the course of the project were all discussed.

2.2 Field survey and sample collection

Two sites (Albert Falls and Midmar Dams in KwaZulu-Natal Province in South Africa) were surveyed and samples of the smut fungus, *Ustilago sporoboli-indici*, attacking *Sporobolus* grasses, *S. pyramidalis* and *S. africanus* were collected (Figure 2.2.1). Majority of the smut infected *Sporobolus* samples were collected from Albert Falls Dam area as the site had more infections than Midmar Dam area. Samples were stored in the refrigerator at 4^{o}C until isolation of the smut fungus.

2.3 Isolation of *Ustilago sporoboli-indici* on solid agar medium

Spores /teliospores of *Ustilago sporoboli-indici* from *S. pyramidalis* and *S. africanus* were gently dusted on water agar plates supplemented with Malt Extract ((0.5g/L) and chloramphenicol (4ml/L at 15mg in 6ml sterile distilled water) to curb bacterial growth. Similarly, 10^{1}-10^{4} dilutions of teliospores were plated for single spore isolation in order to have pure culture. After 22 hours incubation at 22-25^{o}C, spores that germinated both on the dusted and serial dilution plates were

carefully selected under dissecting microscope and subcultured onto a fresh Malt Extract and Potato Dextrose agar plates (Figure 2.3.1).

2.4 Examination of teliospore germination under microscope

Teliospores from infected *Sporobolus* grass were carefully dusted onto solid agar media (previously described under Section 2.3) and incubated for 20-24 hrs at 22-25^{0}C. Germination was examined under Zeiss Axiophot microscope with a camera attached to it and pictures taken [Figures 2.4.1(A-D)]. The purpose of this was to study how the teliospores germinate on agar, (position/direction) of germination, and whether it germinates from one direction or several directions.

2.5 Storage of basidiospores and teliospores

Liquid cultures of the smut fungus (containing basidiospores) were generated by growing the smut fungus in a liquid medium with agar block carrying the smut fungus previously grown on solid agar medium. The liquid medium contained (g/L distilled water): Malt Extract (2 g) and glucose (1.5 g). The liquid medium was distributed in 100 ml volume into 250ml conical flasks. Agar blocks carrying the smut culture were added into flask and incubated in a shaker (water bath) at 250C for 6-10 days. The resulting liquid cultures were stored as follows:

- Sterilise silica gel in McCartney bottles in a furnace at 180^{o}C for 1.5 hrs. Allow to cool.
- Prepare fat-free instant milk powder solution (ratio of 1g in 9 ml sterile distilled water)
- Pipette 5 ml of smut liquid culture and 5ml of sterile milk solution and mix to form a homogeneous mixture
- Pipette 1 ml of the smut liquid culture-milk mixture and dispense slowly into McCartney bottles with silica gel
- Mix thoroughly and label (Figure 2.5.1)
- Keep bottles at room temperature for 24hrs and then transfer bottles into fridge at 4-5^{o}C

Viability of basidiospores stored on silica gel was also evaluated after one month of storage. Pieces of silica gel, on which basidiospores were plated onto water agar supplemented with malt extract powder. Plates were incubated at 25^{o}C in the dark and growth of the smut fungus was observed indicating that the basidiospores survived on the silica gel (Figure 2.5.2). Dry teliospores were also stored in McCartney bottles at -80^{o}C as one of the storage methods.

2.6 Inoculations of *S. pyramidalis* and *S. africanus* with smut cultures (South African populations)

Prior to testing the pathogenicity of the smut isolates against Australian populations of all five of the weedy *Sporobolus* grasses, clumps of *S. pyramidalis* and *S. africanus* were dug, trimmed and transplanted into pots filled with Composted Pine Bark growth medium. Pots were watered daily and once new and fresh shoots emerged, pots were treated with the smut liquid cultures (using a garden spray), kept in a dew chamber at 26^{o}C at 90% rH for 48 hrs. Pots were transferred into a polycarbonate tunnel where they were had watered and monitored for infections. Visible infections were seen after some treatments approximately 8 weeks after inoculations. Infections were notably found on young and fresh leaves.

2.7 Pathogenicity studies of the smut fungus against Australian populations of all five weedy *Sporobolus* grasses

Pathogenicity studies using Australian populations of all five weedy *Sporobolus* grasses began in June 2005. Seeds of all five targeted grasses were germinated in Speedling® 128 trays, with each grass species in a separate tray (Figure 2.7.1). Over 80% germination was achieved for all species except *S. natalensis*, where germination was very poor, with only two seeds germinating from the lots planted. Seedlings were transplanted into pots (one seedling per pot) and drip irrigated (Figure 2.7.2).

Two separate inoculations were performed. Firstly, 8 weeks old seedlings were inoculated with liquid broth cultures (as described under Section 2.5) and secondly, very young seedlings (17 days old) were inoculated with suspensions of basidiospores germinated from teliospores on agar plates overnight at 22-25^{o}C. Inoculated seedlings were placed in a dew chamber at 26^{o}C at 90% rH for 48hrs. Seedlings were removed and kept in a tunnel and drip-irrigated three times a day for 5mins at each watering time. The irrigation water contained soluble fertiliser, NPK 3:1:3 (38) Complete at a rate of 1g/L. The experiment was repeated to confirm results.

Older seedlings inoculated with the smut liquid broth cultures developed infections slower than the younger seedlings inoculated with basidiospores germinated from teliospores. Infections on the older seedlings were first observed approximately 7-8 weeks after inoculations while the younger seedlings developed infections within 4 weeks after inoculations (Figures 2.7.3 – 2.7.7).

Of the five weedy *Sporobolus* grasses, only *S. jacquemontii* did not develop symptoms of infection characteristic of the smut fungus, even after 3 months of inoculation. The same result was obtained in a repeat trial. *S. jacquemontii* is probably a non-host species among the five Australian populations of weedy *Sporobolus* species.

2.8 Infection process of smut fungus on the susceptible *Sporobolus* species: A cytological study

Two weeks old seedlings of *S. pyramidalis*, *S. africanus* and *S. fertilis* were inoculated with the smut fungus by dusting teliospores on leaves of seedlings. Seedlings were kept in a dew chamber, as earlier described, and were transferred into tunnels and drip irrigated. Leaf samples were taken after 14 days and processed as follows for electron microscopy studies.

Sections of leaf samples from inoculated *S. pyramidalis*, *S. africanus*, and *S. fertilis* were fixed in 3% (v/v) glutaraldehyde in cacodylate buffer (0.1M; pH 7.0). After 6hrs of refrigeration at 4^{0}C, the specimens were dehydrated in a graded alcohol-acetone series [10, 20, 50, 70, 80% (v/v)] and twice in 100% (v/v). Dehydrated samples were mounted on copper stubs with double-sided sticky tape and sputter coated with gold-palladium and then kept in a dessicator until examination with Phillips XL30 Environmental Scanning Electron Microscopy (ESEM).

Results of pictures taken from the Electron microscopy studies showed wax removal on leaf surfaces and possible penetration of the smut (Figures 2.8.5 – 2.8.6). Moreover, Figures 2.8.2 – 2.8.4 depicts the smut penetrating the stomatal guard cells. Pictures of germinating spores and possible penetration on all three species were similar.

2.9 Host range trials with ten native Australian *Sporobolus* grass species

Ten (10) native Australian *Sporobolus* grass species: *S. australasicus*, *S. coromandelianus*, *S. laxus*, *S. disjunctus*, *S. scabridus*, *S. contiguus*, *S. creber*, *S. sessilis*, *S. actinocladus* *S. elongatus*, *S. virginicus*, *S. australasicus*, *S. mitchelli* and *S. caroli*, were tested for host susceptibility to the smut fungus. *S. virginicus*, *S. australasicus*, *S. mitchelli* were not tested because their seeds were not germinable, a known charascteristic of *S. virginicus* and *S. mitchelli*. Seeds were germinated in Speedling trays and transplanted into pots, when the seedlings were 3 weeks old. Seedlings were left in the glasshouse for a week before they were treated with suspensions of basidiospores germinated from teliospores on agar plates overnight at 22-25^{o}C. Inoculated seedlings were placed in a dew chamber at 26^{o}C at 90% rH for 48 hrs. Seedlings in pots were removed and kept in a glasshouse and hand watered everyday with tap water. Seedlings received tap water with fertilizer (NPK 3:1:3(38) complete at a rate of 1 g/L) once a week. The number of repetitions depended on availability of seeds of a particular *Sporobolus* species. The results, description of infection and comments on each of the species are presented in Table 2.9.1. Out of the 10 tested for host specificity, four native Australian *Sporobolus* grass species: *S. creber*, *S. elongatus*, *S. sessilis* and *S. scabridus*, developed symptoms of infection caused by the smut fungus. However, *S. creber* and *S. elongatus* were

the two species that were seriously infected (Figures 2.9.1 – 2.9.6), while *S. sessilis* and *S. scabridus* developed minor infections (Figures 2.9.7 – 2.9.10 and also see comments in Table 2.9.1).

2.10 Effect of *Ustilago sporoboli-indici* on flower formation and dry biomass of two weedy and two native *Sporobolus* species

An experiment was done to evaluate the effect of the smut fungus *Ustilago sporoboli-indici* infections on two weedy and two native *Sporobolus* species: *S. fertilis*, and *S. natalensis* and two native species *S. creber* and *S. elongatus*. Seedlings were generated as described under Section 2.9. Seedlings (3 weeks old) of the four grass species were transplanted into 18 cm diameter pots (one seedling per pot). Care was taken to choose seedlings of approximately the same height and size. Forty pots were planted with seedlings for each grass species. Twenty pots with seedlings were treated and served as the test, while the remaining untreated 20 pots with seedlings served as controls. Inoculations were carried out, as described under Section 2.9. Seedlings were hand watered daily and received water with fertilizer once a week as described under Section 2.9. Number of tillers with flowers and dry biomass of the grasses in each pot were determined 90 days after inoculation (90 days post inoculation period). Summary of results and statistical analysis are presented in Table 2.10.1.

Table 2.9.1 Results, description and comments on host range trials of 13 native Australian *Sporobolus* grass species against the smut fungus, *Ustilago sporoboli-indici*

Name of *Sporobolus* species	Infection on species	Description of infection	Comments
actinocladus	NO	No visible infection seen on all plants/seedlings treated with the smut fungus	First, second and third trials produced the same results. Final and fourth trial confirmed the results of first, second and third host testing trials of this species.
austrasilacus	NOT DETERMINED	Seeds did not germinate. Hence, host testing on this species was not successful.	All attempts to germinate the seeds in the greenhouse failed.
caromandelianus	NO	No infections on all seedlings inoculated with the smut fungus, after 70 days.	Two repeated trials produced similar results. No infections seen on inoculated seedlings.
caroli	NO	No infections seen on all seedlings treated with the smut fungus.	Three repeated trials produced same results which confirmed the first and second trials.
creber	YES	Seedlings treated with the smut fungus developed infections typical of the smut. Tillers of plants that were infected developed loop/whip with teliospores. Seriously infected young seedlings rarely develop flowers and in situations where flowers develop, they are malformed (See figures 2.9.1 – 2.9.8)	One or more seedlings in 50-60% of the pots developed infection when treated with the smut. First, second third and fourth trials of host testing showed similar results. Probably the most seriously affected species among the native Australian species.
contiguus	NO	No visible infection seen on seedlings treated with the smut fungus.	Repeated trials produced similar results. No infections were seen.
disjunctus	NO	No visible infections seen on all seedlings inoculated with the smut fungus.	Repeated trials produced similar results. No infections were seen.

Name of *Sporobolus* species	Infection on species	Description of infection	Comments
elongatus	YES	Seedlings inoculated with the smut fungus were infected. Infections were similar to that of *S*. *creber*. Formation of teliospores in tillers and deformed flowers when severely attacked.	One or more seedlings in 45-50% of pots developed infections when treated with the smut fungus. Results of first, second and third host testing trials were similar. There is no doubt that *creber* and *elongatus* are susceptible to the smut fungus.
mitchellii	NOT DETERMINED	Seeds did not germinate. Hence, host testing on this species was not successful.	All attempts to germinate the seeds in the greenhouse failed.
virginicus	NOT DETERMINED	Seeds were unable to germinate. Hence, host testing on this species was not successful.	All attempts to germinate the seeds in the greenhouse failed.
sessilis	YES, MARGINALLY	First host testing trial had ONLY three leaves infected, while a repeat trial showed only a leaf infected with the smut with formation of teliospores. A third repeat trial had more than three leaves on three different plants inoculated with the smut fungus. Infections however were not damaging compared to that of *S*. *creber* and *S. elongatus*.	Only seedlings of one pot showed any infection (three leaves) during the first trial. One seedling in one pot developed any infections (one leaf) in the repeat trial. Three seedlings were infected in the third repeat trial with more than three leaves infected. Results of fourth trial confirmed that an infection on *S*. *sessilis* is very marginal.
scabridus	YES, MARGINALLY	Only one leaf was infected. Third repeat trial showed infection only on two leaves on plant in only one pot. Infections seem more localized than spread on infected leaves.	Seedlings in only one pot were infected. It is also worth noting that no infections were seen during the first trial. However, two leaves were infected during the third repeat trial. Fourth trial showed 2-3 leaves with infections in two separate seedlings.
laxus	NO	No visible infection seen on all plants/seedlings treated with the smut fungus	First, second, third and fourth trials showed no infections.

Table 2.10.1 Analyses of results obtained from the effect of damage caused by the smut fungus of four *Sporobolus* grass species (two native and two alien invasive species).

Treatments	Mean no. of tillers with flowers (mean from 20 plants)	Mean dry biomass [g] (mean of 20 plants)	Total no. of tillers with flowers	Total number of tillers with flowers infected	Percentage of tillers with flowers infected
S. creber	15[a]	10.57[a]	299	63	21.1
Control	11.2[a]	8.91[a]	225	–	–
P-value	0.062	0.234	–	–	–
Significance	ns	ns	–	–	–
lsd	4.49	2.38	–	–	–
S. elongatus	6.20[a]	13.87[a]	124	15	12.1
Control	7.10[a]	13.38[a]	142	–	–
P-value	0.343	0.69	–	–	–
Significance	ns	ns	–	–	–
lsd	1.94	2.55	–	–	–
S. fertilis	6.85[a]	13.46[a]	127	18	14.2
Control	7.50[a]	13.75[a]	150	–	–
P-value	0.288	0.737	–	–	–
Significance	ns	ns	–	–	–
lsd	1.25	1.78	–	–	–
S. natalensis	5.35[a]	16.23[a]	107	3	2.8
Control	5.10[a]	13.96[a]	102	–	–
P-value	0.754	0.111	–	–	–
Significance	ns	ns	–	–	–
lsd	1.65	2.85	–	–	–

ns = Not Significant

Values with the same superscript are not significantly different (P ≥ 0.05)

With respect to the number of tillers with flowers formed and dry biomass, results indicate that there were no significant differences between each of the four *Sporobolus* grass species treated with the smut fungus and their respective untreated controls (Table 2.10.1).
In terms of the number of tillers with flowers formed on treated grass plants, *S. creber* is the native species which had the highest numbers of tillers with flowers formed and at the same time recorded the highest percentage (21.1%) of tillers with flowers infected with the smut (malformed) (Table 2.10.1). This was followed by *S. fertilis*, *S. elongatus* and *S. natalensis* in that order. Hence the descending order of the percentage infected tillers with flowers formed is *S. creber* > *S. fertilis* > *S. longatus* > *S. natalensis*.

Comparison between all the four grasses treated with the smut fungus without their respective controls indicate that there were highly significant differences between the numbers of tillers with flowers formed , percentage of tillers with flowers infected and dry biomass (Table 2.10.2).

Table 2.10.2. Comparison of all four *Sporobolus* grass species without their respective controls

Treatments	No. of tillers with flowers (mean of 20 plants)	Percentage of tillers with infected flowers
S creber	15.0[b]	21.1[b]
S. elongatus	6.20[a]	12.1[a]
S. fertilis	6.85[a]	14.2[a]
S. natalensis	5.35[a]	2.8[a]
P-value	0.001	0.001
Significance	***	***
lsd	2.63	1.09

*** Significantly different at P ≤ 0.001

Values with the same superscript are not significantly different (P ≥ 0.05)

3. Discussion

3.1 General Remarks

The results obtained from pathogenicity trials against the five weedy *Sporobolus* grasses indicate that four (4) species: *S. pyramidalis*, *S. africanus*, *S. fertilis* and *S. natalensis*, out of the five grasses, were susceptible to the smut fungus, *Ustilago sporoboli-indici*. Only *S. jacquemontii* did not show any signs or symptoms of infection typical of the smut fungus during the duration of the trial and even after extended periods of time. At the time of writing this report, seedlings of *S. jacquemontii* inoculated three months ago have still not shown any signs of infection. This was also observed during the first pathogenicity trial. Absence of infection/symptoms in *S. jacquemontii* after prolong period of inoculations with the smut fungus indicates that *S. jacquemontii* is probably not a non-host to the smut fungus.

Basidiospores or inoculum obtained from washed agar plates dusted with teliospores and germinated overnight were more effective and quicker to cause symptoms of infection than inoculum generated from continuous or submerged broth culture.

Young seedlings developed disease symptoms quicker to infection than older seedlings, irrespective of the type of inoculum used. Happily, teliospores collected from *S. pyramidalis* caused infection on all four susceptible *Sporobolus* grasses and *vice versa* for teliospores collected from *S. africanus*. Hence, it does not matter from which *Sporobolus* host grass teliospores are sourced or collected from, for infection/pathogenicity trials.

The smut fungus is slow to cause infections. In most cases, infections are not uniformly spread out in all treatments. For the smut fungus to be considered as a possible classical biological control agent, there is a need for more work to be done on how to improve the performance of the fungus. Trials using very low doses of 2,4-D and Round-Up to increase the susceptibility of the alien species to the smut are needed. Sublethal doses of herbicide suppress or inhibit the biosynthesis of compounds such as phytoalexins, enzymes or hormones, making the plant more susceptible to infection by pathogens. Herbicides such as glyphosate and 2,4-D have been used in a

weed/pathogen system to enhance susceptibility of weeds, hence improving the efficacy of the classical biological control agent (Charudattan, 1986; Sharon *et al*., 1992). We believe that this approach is technically and economically feasible, and would like to include it in future trials.

Host range trials using the 10 native Australian *Sporobolus* grass species indicated that four of the 10 native *Sporobolus* grass species developed symptoms typical of the smut fungus. Infections on two of the four species: *S*. *creber* and *S*. *elongatus*, were more damaging which affected the flowers formed (malformed) in some of the plants treated with the smut fungus (Figures 3.1.1 – 3.1.4 and Figures 3.1.5 – 3.1.8). Figures 3.1.9 – 3.1.12 and Figures 3.1.13 – 3.1.16 also compare the treated *S*. *fertilis* and *S*. *natalensis* and their respective untreated controls. *S*. *fertilis* appears to be more affected than *S*. *natalensis*. However, the other infected two native species: *S. sessilis* and *S. scabridus*, developed infections which were not as damaging compared to the infections developed by *S*. *creber* and *S*. *elongatus*. Hence, *S*. *creber* and *S*. *elongatus* appears more susceptible to the smut fungus, unlike *S. sessilis* and *S. scabridus*, which appears to be partially susceptible to the smut.

With 4 of the 10 native Australian *Sporobolus* grass species infected (2 clearly susceptible, 2 marginally susceptible), it therefore means that the ideal result from these trials did not occur. This would have been complete resistance by all native species to the smut, and hence a zero risk to native *Sporobolus* species, if the smut fungus were to be used as a classical biological control agent for the five weedy *Sporobolus* grass species in Australia. Although this is a matter of concern, the primary use of the smut fungus as a classical biological control agent in Australia would be to prevent worthless exotic *Sporobolus* grasses dominating palatable grass species, of various genera, some of which are also exotic.

The issue revolves around the relative susceptibility of the two groups of grasses, native and invasive, and their respective distribution and density. Plant disease epidemiologists have shown that whilst host susceptibility is important, the host population distribution and density are the key factors in determining whether a pathogen will spread in a host crop (Zadoks and Schein 1979) (see Appendix 1 for a detailed explanation).

The paradigm for disease spread is that:

1. if the host plants are resistant, then there will be no disease spread
2. if the host plants are susceptible, AND they are in monocultural stands, then disease spread will occur
3. if the host plants are susceptible, AND they are in monocultural stands in high densities, then disease spread will occur, even more rapidly
4. if the host plants are susceptible, BUT they are distributed widely, as individual plants mixed with other non-host plants , then disease spread will NOT occur, or will occur at low rates. (what happens is that spores of the pathogen largely fall on non-hosts and therefore die).

The question on *Sporobolus* then is: What is the distribution and density of the native species versus the invasive species in Australia?

Distribution of Native and Invasive *Sporobolus* Species in Australia

In my experience, weedy *Sporobolus* species are often found with little or no native *Sporobolus* present (NSW North coast).

I have seen a small percentage of infestations, often newer or sparse infestations on native and naturalised grassland, where both the weedy and native species are found together. In the latter case, there is almost always more weedy *Sporobolus* than native.

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We therefore propose that, in general:

1. Alien invasive *Sporobolus* grass species form large clusters of genetically and morphologically uniform plants, growing closely together, resulting in dense, monocultural stands, with stable microenvironments, which would favour infection by the smut fungus, and its spread and disease development. With fairly large, uniform populations of potential hosts (alien invasive *Sporobolus* grass species), growing close together in space and time, there would be ample opportunity for the smut fungus to attack a large number of invasive *Sporobolus* plants.

2. Native *Sporobolus* species grow in dispersed, patchy distributions of low density. Therefore, the number of infections, and the spread of the smut fungus, would be very slow on these species, even if they are susceptible to the smut fungus.

3. The net result would be that the smut fungus should attack the invasive species much more vigorously than the native species, simply because of distribution and density patterns. This would ensure that there is less infection and relatively good protection of the native species, most of which are fully resistant to the smut fungus.

Ideally, the smut fungus would attack the invasive species vigorously enough to stop their spread in Australia, and to reduce their presence to a patchy, low density occurrence in a stable pathosystem. Their further growth and spread in Australia should be kept in check by the smut fungus. At the same time, the smut should have no significant consequence on the native *Sporobolus* species.

Whether the smut fungus should be taken further as a potential biological control agent for invasive *Sporobolus* species in Australia is based on the considerations of both its potential to control the invasive species effectively, and the risks it poses for the susceptible native species.

This complex decision rests on the shoulders of the stakeholders involved in this project, based on the information provided in this report and after careful analyses of the ecological consequences the smut might pose to Australian grasslands.

5. Papers/posters in preparation for conferences and publication in refereed journal in 2006

Conference: Biocontrol Conference (Montpellier, France) 22-27th April 2007
1. Potential of *Ustilago sporoboli-indici* for biological control of five invasive *Sporobolus* grasses in Australia

Paper (s) in preparation for refereed journal:
1. Evaluation of *Ustilago sporoboli-indici* as classical biological control agent for invasive *Sporobolus* grasses in Australia.

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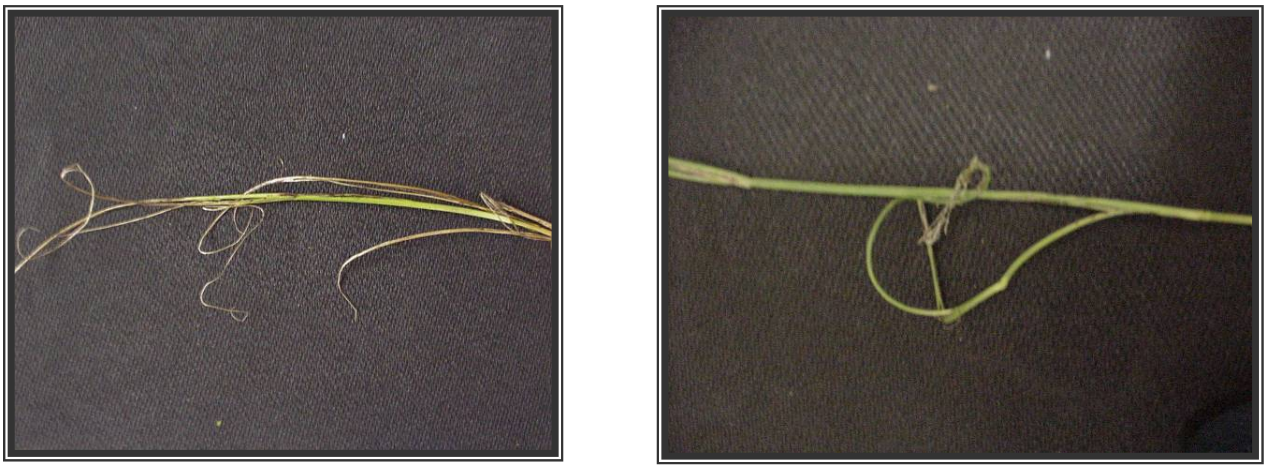


Figure 2.2.1. Sample of smut-infected *Sporobolus* grass collected from Albert Falls Dam resort

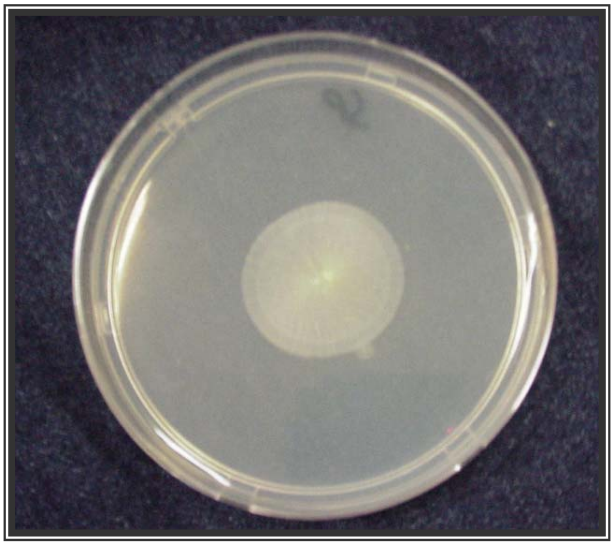
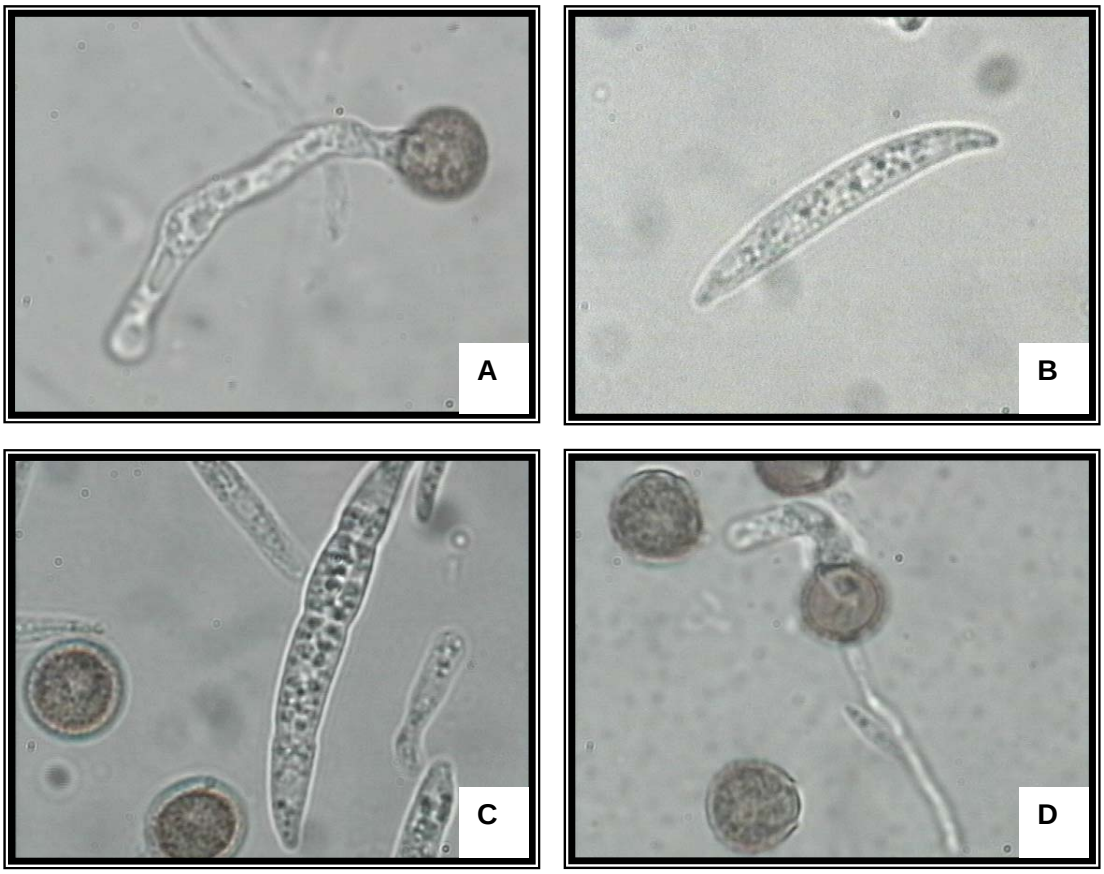


Figure 2.3.1. A culture of the smut (*Ustilago sporoboli-indici* growing on water agar supplemented with 0.5 g/L malt extract and chloramphenicol.



Figures 2.4.1 A-D illustrates teliospore germination and basidiospore?(s) on solid agar medium

Figure 2.5.1 Basidiospores stored on silica gel

Figure 2.5.2 Survival (viability) of basidiospores after storage on silica gel

Figures 2.7.1 (left) and 2.7.2 (right) Twenty-two days old *Sporobolus* seedlings in Speedling® 128 tray (left) transplanted into pots (right).

Figure 2.7.3: Infections on old *Sporobolus pyramidalis* seedlings (circled) with formation of teliospores (black)

Figure 2.7.4 (left) and 2.7.5 (right): Infections on young *S. pyramidalis* seedlings (left) and on *S. africanus* seedlings (right)

Figure 2.7.6 (top) and 2.7.7 (bottom): Infections on young *S. natalensis* seedlings (left) and on *S. fertilis* seedlings (right)

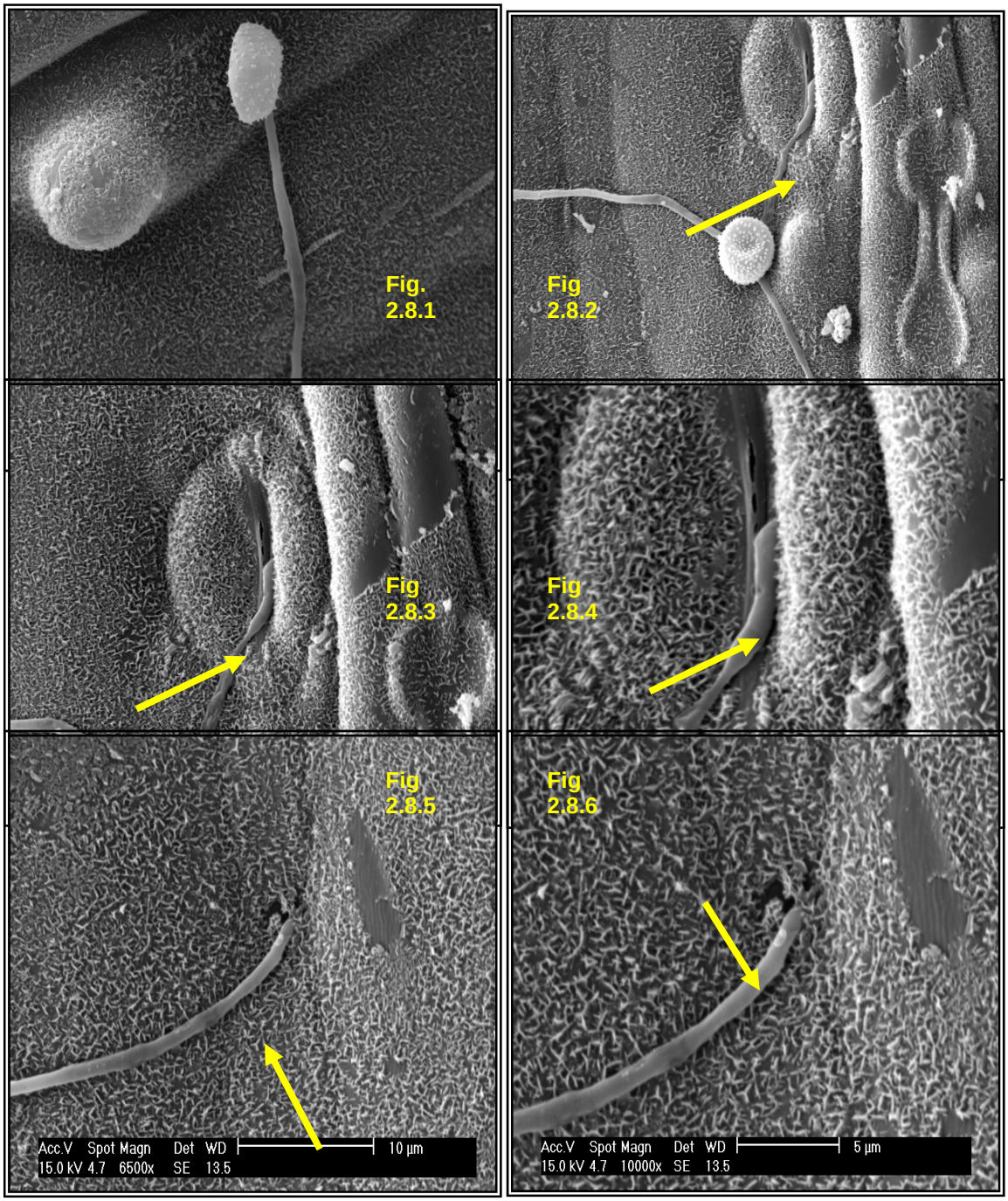


Figure 2.8.1: Germination of smut teliospore on *Sporobolus* grass leaf surface after 24 hrs; Figure 2.8.2: Possible penetration through the guard cells of stomata (arrowed); Figure 2.8.3 and 2.8.4: 5000 and 10,000X magnification of Figure 2.8.2 showing possible penetration of smut through the stomata guard cells; Figure 2.8.5: Wax removal on *Sporobolus* leaf surface and possible penetration through epidermis? (arrowed); Figure 2.8.6 10,000X magnification of Figure 2.8.5.

Figure 2.9.4

Figure 2.9.5

Figure 2.9.6

Figure 2.9.4: Infection caused by the smut fungus on *S. elongatus* causing death of seedling leaves; Figure 2.9.5: Close view of infections on *S. elongatus* (circled); Figure 2.9.6: Close view of severe tiller destruction caused by the smut fungus on S. *elongatus* caused by the smut on *S. creber* with the formation of teliospores (dark in colour).

Figure 2.9.8

Figure 2.9.9

Figure 2.9.10

Figure 2.9.8 Infections as seen on *S. sessilis* during the repeat of the first host range testing of the smut fungus and Infections on *S. scabridus* during the first and second host range testing with the smut fungus (Figure 2.9.9 and Figure 2.9.10 respectively).

Figure 3.1.9

Figure 3.1.10

Figure 3.1.11

Figure 3.1.12

Figure 3.1.9: Damage on *S. fertilis* due to the smut fungus infection – dead leaves, compared to untreated *S. fertilis* plant (Figure 3.1.10). Figure 3.1.11 represents damage on flowers caused by the smut fungus, compared to healthy flowers of untreated *S. fertilis* plant (Figure 3.1.12)

Figure 3.1.13

Figure 3.1.14

Figure 3.1.15

Figure 3.1.16

Figure 3.1.13: Damage on *S. natalensis* due to the smut fungus infection, compared to untreated *S.* natalensis plant (Figure 3.1.14). Figure 3.1.15 represents damage on flowers caused by the smut fungus, compared to healthy flowers of untreated *S. natalensis* plant (Figure 3.1.16).

9.3 Appendix 3: Report on stem wasp by PPRI, South Africa

FINAL REPORT TO QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES & FISHERIES

APRIL 2007

The biology, impact and host range of *Tetramesa* sp. (Hymenoptera: Eurytomidae), a potential biological control agent for *Sporobolus pyramidalis* (P.Beauv) (Cyperales: Poaceae) in Australia.

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ABSTRACT

Sporobolus pyramidalis, *S. africanus* and *S. natalensis* were accidentally introduced to Australia from Africa and have the potential to invade 223 million hectares. Mechanical and chemical controls are largely ineffective and expensive, hence the search for potential biocontrol agents in southern Africa. The two most promising potential agents found in surveys in southern Africa in 2001/2002 were an undescribed *Tetramesa* species (Hymenoptera: Eurytomidae) and a smut, *Ustilago sporoboli-indici* (Ustilaginales: Ustlaginaceae). The stem-boring eurytomid was found to be particularly damaging at some sites during initial surveys. Of the 144 *S. pyramidalis* culms randomly collected at a particular site in 2002, 33% were infested with *Tetramesa* sp. larvae and/or pupae. The inflorescences and culms of 60% of these infested culms were malformed. These preliminary results warranted further investigation of *Tetramesa* sp. as a potential biocontrol agent in 2006/2007. Studies were undertaken to determine its impact, phenology and biology with the primary goal to establish a laboratory culture. Unlike the results obtained in 2001/2002 larval feeding appeared to have no significant impact on culm or inflorescence length. More larvae were found in dissected culms in autumn and winter while pupae were more common in summer. This supported the results of laboratory trials, which indicated that *Tetramesa* sp. entered larval diapause in winter. Although manipulating temperature and day length in laboratory situations could break diapause, a laboratory culture on potted grasses could not be established. This paper gives a detailed account of the work undertaken in order to determine the impact of *Tetramesa* sp. on *S. pyramidalis* and to establish a laboratory culture.

INTRODUCTION

Species in the *Sporobolus indicus* complex, like *S. africanus* (Poir) Robyns & Tournay, *S. pyramidalis* P. Beauv. and *S. natalensis* (Steud.) Dur. & Schinz., were accidentally introduced to Australia from Africa and have subsequently become invasive, posing a major threat to the environment and livestock production. It has been estimated that this complex of invasive species could invade approximately 223 million hectares in Australia (Department of Natural Resources & Mines, 2001). Various control methods have been employed in Australia including the use of fire, herbicides, over-seeding with indigenous grass species and mechanical control (digging, hand pulling and cutting) (Vogler & Bahnisch, 2006). These methods are costly in terms of time and labor and have not been very successful, resulting in the search for potential biocontrol agents in southern Africa in 2001 and 2002. Ninety sites in South Africa, Swaziland and Botswana were surveyed for potential biological control agents resulting in the selection of a pathogen (*Ustilago sporoboli-indici* (L. Ling) (Ustilaginales: Ustlaginaceae) and a wasp (*Tetramesa* sp.) (Hymenoptera: Eurytomidae) for further study (Witt and McConnachie, 2003). In this document we report on aspects of the phenology, biology and impact of *Tetramesa* sp. on *S. pyramidalis*.

Biological control of alien invasive plants

Invasion of alien species across the planet is the second biggest threat to biodiversity, following habitat loss (Corey, 2000). These invasions have resulted in major ecological and economic impacts to natural environments as invasive species compete with native plants for nutrients, water and light. Invasive plants not only alter the functioning of ecosystems by reducing the amount of water available to native plants but they also decrease plant productivity and diversity (Naeem *et al*., 1999). Conventional control and eradication efforts are costly and in many cases ineffective (Anderson *et al*., 1996; Mack *et al*., 2000).

In classical biological control, biocontrol agents, such as insects, mites and/or pathogens, are deliberately introduced from the weed's region of origin into the introduced range (van Wilgen *et al*., 2004). These are not intended to eradicate the weed but merely to reduce plant vigour and/or flower and seed production. The agents are said to be successful when they reduce the population density and rate of spread of the weed (Zimmerman *et al*., 2004).

The biggest concerns with regard to biological control are potential non-target affects. Although some introductions have adversely affected native plant species, most of these were predicted prior to the agents being introduced (Thomas & Willis, 1998). With improved host specificity testing through centrifugal phylogenetic testing, the potential risks have been further reduced (McEvoy, 1996). Post-release evaluation studies have also improved our understanding of the effectiveness of agents and have contributed to our predictive abilities with regard to agents prior to release.

The biological control of invasive plants has been practiced since 1863, but the first biocontrol agent, *Dactylopius ceylonicus* (Green), was only introduced to South Africa in 1913 to control Brazilian cactus, *Opuntia vulgaris* (Olckers, 1999). The science of biocontrol has been growing ever since, with over 370 successful biocontrol agents introduced worldwide (Zimmerman & Klein, 1999). Because of the co-operation with other countries on projects of international significance and more recently as a result of additional funding from the "Working for Water" (WfW) program of the Department of Water Affairs and Forestry (DWAF) (Zimmerman & Neser, 1999), South Africa has a strong culture of weed biocontrol. More than 85 species of biocontrol agents have been released onto 47 weed species, making South Africa the third most active country in the field after the USA and Australia (Olckers, 1999). Australia has also brought a number of invasive species under good biological control, including numerous *Opuntia* spp., *Chondrilla juncea* (Skeleton weed), *Salvinia molesta* (Kariba weed) and *Cryptostegia grandiflora* (Rubber vine) (Julien *et al*., 2006).

Grasses as targets for biological control

Grasses are probably one of the most useful family of plants to people. The cereal grasses, namely rice, wheat, maize, barley, oats, sorghum and millet form a third of the world's diet. They also provide grain and forage for animals that provide meat and milk for human consumption (Burton, 1993). At the same time the family Poaceae also has the highest percentage of weedy species of any plant family with more than 22% of all grass species being classified by Randall (2002) as weeds. In fact, the top five species of weed worldwide, based primarily on the impact they have in agriculture in control costs and yield reduction are in the Cyperaceae or Poaceae, with *Cyperus rotundus* L. being the world's worst weed (Holm *et al*. 1977). Despite the fact that so many grass species are invasive there have been very few biocontrol programs initiated against species in the Poaceae.

The lack of biocontrol programs against grasses is probably as a result of the fact that grasses are perceived as lacking specific herbivores, and as being too similar in morphology, physiology and ecology to crop species (Witt and McConnachie, 2003). In addition, invasive grasses are often also overlooked as targets for control because they are generally not noticed in native grasslands, especially if they have many native congeners, and their impact is therefore seen as negligible. They are generally also harder to identify when compared to dicotyledonous plants (Milton, 2004). Despite this there have been a number of projects in the recent past in which grass species have been targeted for biocontrol. Surveys of arthropods associated with grasses have indicated that many of them are monophagous or oligophagous and extremely damaging (Witt and McConnachie, 2003).

The target – *Sporobolus pyramidalis*

Unlike most other invasive taxa most grass species were accidentally introduced. Examples include the annual *Bromus tectorum* (L.) in the USA (DiTomaso, 2000); the perennial *Nasella trichotoma* (Nees) in South Africa and *Cenchrus echinatus* (A.S. Hitchc.) in Hawaii (Zavaleta *et al*., 2001). They were generally introduced as seed contaminants, particularly in Australia which imported vast quantities of grass seeds from Africa in order to improve pasture production (Milton, 2004); re-vegetate cleared land; and reduce overgrazing of natural pastures. Weedy grasses are particularly costly in that they can alter ecosystems, reduce the value of pasture areas in agriculture and decrease biodiversity (Queensland Department of Natural Resources & Mines, 2001).

In Australia the following grasses of African origin, are invasive (Nadolny, 2005; Burton, 1993; Corey, 2000):

- Giant rats tail grass (*Sporobolus pyramidalis*)
- Buffel grass (*Cenchrus ciliaris*)
- African lovegrass (*Eragrostis echinochloidea*)
- Coolatai grass (*Hyparrhenia hirta*)
- Gamba grass (*Andropogon gayanus*)
- Mission grass (*Pennisetum polystachion*)
- Para grass (*Brachiaria mutica*)

Invasive *Sporobolus* species have been recorded in New Guinea, Malaysia, Sri Lanka, India, Japan and Korea. Several other closely related species have been found in South America, Central America and southern USA. Some *Sporobolus* species are described as serious weeds in southern USA having invaded more than 6.5 million ha. Many ecotypes of the grass have also been found on islands in the Pacific and Indian oceans and in the Caribbean (Pomery, 2000).

There are approximately 160 *Sporobolus* species in tropical and subtropical areas. Of the 21 *Sporobolus* species in Australasia, 13 are endemic (Simon & Jacobs, 1999). However, the recognition of many of these species, especially those in the *S. indicus* complex are difficult because of the morphological intergradation in the genus (Simon & Jacobs, 1999). Species in the *S. indicus* complex occur on all soil types and generally in areas with high rainfall. *Sporobolus pyramidalis* occurs throughout tropical Africa and Madagascar, Mauritius and Yemen while *S. africanus* and *S. natalensis* are found from southern Africa as far north as Ethiopia.

Sporobolus species are generally aggressive, robust, perennial grasses, which have low palatability when mature and are mechanically difficult to control. The tussocks are distinct and extremely well rooted making them difficult to remove mechanically. *Sporobolus* species can also be extremely fast growing, taking a minimum of three months to mature (Department of Natural Resources & Mines, 2001). Seed viability is 90-100%, with as many as 150,000 seeds/m^2 in infested pastures and a seed bank which may remain viable for as long as 10 years (Department of Natural Resources & Mines, 2001). The mature seeds are dispersed easily when damp because they become sticky and attach to animal fur, cars and machinery. It also can spread through animal faeces, flowing water and the very reason for Australia's problem – contaminants in pasture seed (Clifford, 1959). They can reduce pasture productivity and out-compete beneficial pasture grasses especially following overgrazing or soil disturbance (Walton, 2001). This results in a decrease in the biodiversity of indigenous grass species (Department of Natural Resources & Mines, 2001). *Sporobolus* species tough leaves can also increase teeth wear in cattle and horses when grazing (Walton, 2001).

Sporobolus pyramidalis is distributed across the eastern savannas of southern Africa (Fig. 1) and occurs predominantly in disturbed areas where it forms dense patches. Because this species is

largely unpalatable farmers in southern Africa attempt to control it by ploughing it up and replanting the pasture or digging out scattered tussocks (Bray, 2003). Similar to the situation in Africa invasive *Sporobolus* species occur over a wide range of soils and conditions in Australia (Department of Natural Resources & Mines, 2001) and these grasses have the potential to spread further unless controlled (Fig. 2). Some farmers in Australia have recorded losses in carrying capacity of their stock and decreased production ranging from 10−80%, depending on the density of infestations (Department of Natural Resources & Mines, 2001). Stock on invaded pastures can take an additional 12 months to reach equal weights compared to those feeding on uninvaded pastures (Department of Natural Resources & Mines, 2001).

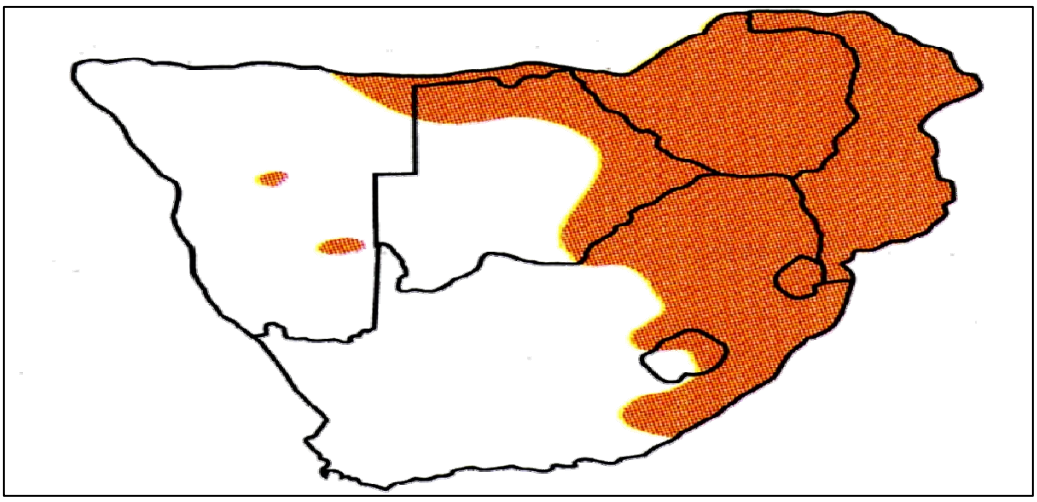

Figure 1: The distribution of *Sporobolus pyramidalis* in Southern Africa (Russel *et al*., 1990).

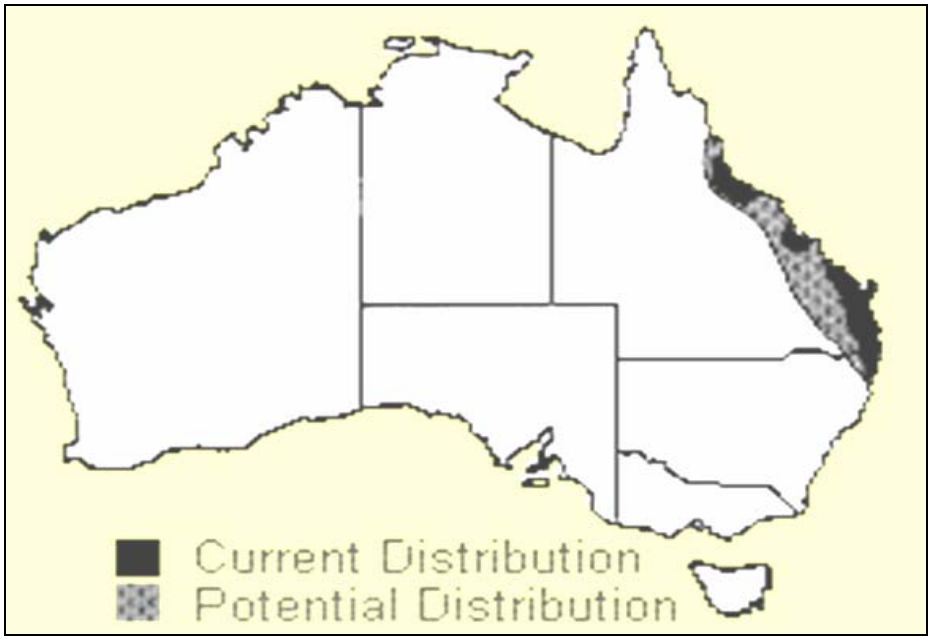

Figure 2: The current and potential distribution of *Sporobolus pyramidalis* in Australia (Department of Natural Resources & Mines, 2001).

The agent – *Tetramesa* sp.

Species in the family Eurytomidae exhibit extremely diverse host relations, some being parasitic, others phytophagous, feeding on seeds or forming galls while other species pass through a phytophagous and parasitic phase during their development (Pitkin, 2004). Phytophagous species in southern Africa are often reared from the seeds of legumes and grass stems, and a species of *Eurytoma* is associated with galls found on the leaves of *Erythrina* species (Scholtz & Holm, 1985). The main diagnostic features of this family include a robust to elongate body that is strongly sculptured and uniformly black, but also yellowish to brown, measuring about 1.4-6.0mm in length (Pitkin, 2004).

Figure 3: Illustration of a *Tetramesa* species.

Many species in the Eurytomidae are known to be host specific. Martinez *et al*.(1999) found 18 different species of eurytomids in 10 sympatric species of grasses, with no species occurring in more than one species of grass. Despite this proof of host specificity not many eurytomids have been used as biocontrol agents. The most frequently quoted use of a eurytomid as a biocontrol agent is that of the bud-galling wasp, *Trichilogaster acaciaelongifoliae* (Frogatt), which was introduced for the control of *Acacia longifolia* in South Africa (Dennill & Donnelly, 1991).

Tetramesa sp. (Fig. 3) was selected as a biocontrol agent as a result of intensive surveys undertaken on various *Sporobolus* species by staff from the South African Field Station, Queensland Department of Natural Resources & Mines in 2001/2002 (Witt & McConnachie, 2003). A host of other insects were also collected during these surveys, most of them pollen-feeders, while immature cicadellids, aphids and moth larvae were collected on the leaves and inflorescences. Unfortunately, none of these other than *Tetramesa* sp. were seen as having potential as biocontrol agents (Palmer *et al.,* 2003; Witt & McConnachie, 2003).

Tetramesa sp. was thought to be a promising agent because preliminary studies in 2001/2002 indicated that larval activity in the culms significantly reduced the length of culms. Larval feeding in the culms of *S. pyramidalis*, was assumed to lead to the malformation or stunted growth of the inflorescence (Witt & McConnachie, 2003). It was assumed that there was a selective advantage to having tall culms in that they may contribute to increased dispersal away from the mother plant – shorter culms may therefore contribute to a reduction in the distance dispersal of seeds or even pollination success. Larval feeding in the culms may also have disrupted the flow of nutrients from the roots to the inflorescence, which may have affected seed production and seed viability. Preliminary studies indicated that there was no significant difference in seed weight between uninfested and infested culms. Based on this evidence one could assume that larval feeding had no impact on seed development but this would be premature, as larval feeding may still have affected the number of seeds on each inflorescence and may very well have reduced seed viability.

Although eurytomids are not known to kill plants they can reduce crop yields substantially. *Eragrostis teff* (Zucc.) Trotter was introduced to the United States where it was attacked by the stem-boring eurytomid *Eurytomocharis eragrostidis* (Howard), causing a reduction in forage yields of over 70% in one year (McDaniel and Boe, 1990). Contrary to what was found in our initial studies, Spears and Barr (1985) also found that *Tetramesa* spp. reduced seed weight in *Aristida longiseta* Steud., *Sitanion hystrix* (Nutt.) J.G. Smith, *Sporobolus cryptandrus* (Torr.) A. Gray and *Stipa comata* Trin. & Rupr. by 47, 33, 46 and 60%, respectively. This resulted in a reduction in seed germination for all four species with as many as 99% of seeds of *A. longiseta* not germinating (Spears and Barr, 1985). Stem borers other than eurytomids are known to be pests of important agricultural crops including sugar, rice, maize and sorghum. Lepidopteran stem borers cause the most damage to these crops. *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) is the most destructive stem borer of sugarcane in South Africa (Keeping & Meyer, 2002) and also attacks maize in West and Central Africa (Ajala *et al*., 2001). One of the main advantages of using stem borers as biocontrol agents is that they are well protected, especially from parasitoids, in their immature stages (Monetti *et al*., 2003).

MATERIALS AND METHODS

Sporobolus pyramidalis culms together with their inflorescences were collected once per month, over the entire study period (February 2006 – February 2007), on a farm near Modimolle in Limpopo Province, South Africa. Approximately 60 culms, selected randomly from individual plants, were cut-off at the base, just above the crown and brought back to the laboratory. In addition four other grasses (*Cymbopogon* sp.*, Sorghum* sp.*, Hyparrhenia* sp. and *Pennisetum* sp.), growing in close proximity to *S. pyramidalis*, were also collected at the same time in order to determine the field host range of *Tetramesa* sp. collected on *S. pyramidalis*.

The length of all inflorescences and culms were recorded. Each culm was then inspected for emergence holes and dissected in order to determine if there were any larvae, pupae or pre-adults present in the culm. The impact of larval feeding could then be ascertained by comparing the culm and inflorescence length of healthy or uninfested culms with that of infested culms (larvae, pupae, pre-adults or emergence holes present). A large number of grass culms which were thought to be infested because they were shorter and in some cases malformed were placed in large cages in the hope that larvae and pupae would continue their development and later emerge as adults.

Larvae, pupae and pre-adults were also removed from dissected culms and placed in petri-dishes together with moist filter paper in order to maintain humidity. Petri-dishes with the various life stages were initially placed in a controlled environment chamber at a temperature ranging between 20 and 22°C and a photoperiod of 12 h D: 12 h N. Petri-dishes were also placed in an incubator with a photoperiod of 14 h D: 10 h N and temperatures during the simulated day and night period of 30ºC and 20ºC respectively, in order to break larval diapause. Some larvae were also placed in an artificial diet in paper straws and in some cases the artificial diet was merely placed in the petri-dish near the larvae. The advantage of an artificial diet is that it enables one to rear insects throughout the year, irrespective of the phenology of the host plant. Great success has been obtained with the rearing of a host of stem-boring insects on artificial medium (Singh, 1975). Kastings and McGinnis (1958) reported on the mass rearing of the wheat stem sawfly on an artificial diet and Villacorta *et al*. (1971) developed artificial oviposition sites for the wheat stem sawfly. *Tetramesa* sp. larvae were provided with artificial diets used in the mass rearing of *Chilo partellus* and *Heliothis armigera*. An artificial diet used in the mass rearing of *Eldana saccharina* by the South African Sugar Research Institute (SASRI) was also used.

All emerging *Tetramesa* sp. adults were placed in cages on potted *S. pyramidalis* plants. The plants had culms varying in size and age to ensure that the females were exposed to suitable oviposition sites. All the life stages were monitored and the time taken to change from one stage to another was noted.

RESULTS AND DISCUSSION

Biology

Very few adults emerged from grass culms collected in the field and placed in large cages. The grasses dried out extremely rapidly and adults could not emerge through the hardened cuticle of the culm once it had dried out. Attempts to rear the larvae on artificial medium also proved to be problematic as a result of fungal contamination of the growth medium. In addition, it did not appear that the larvae were feeding anyway. This was confirmed when larvae were placed in petri-dishes together with some moist filter paper in order to enhance humidity levels. Although artificial medium was placed in some petri-dishes larvae did not move closer to it in any of the replicates. Larvae with and without access to growth medium managed to survive equally well. This led to the conclusion, as we hypothesized, that *Tetramesa* sp. larvae enter larval diapause, probably at the last instar stage and do not need to feed in order to continue their development during this period.

Of the 51 larvae dissected out of culms collected in May 2006, for example, 20 pupated and two adults emerged 25 days after the culms were collected in the field. Unfortunately 15 larvae died during that period with the remaining 14 larvae still looking healthy. The 15 larvae that died may have been injured when they were removed from the culms – there is also the possibility that they were earlier instars and required a food source other than artificial medium to survive. The ones that did survive support our contention that late instar larvae can survive for periods of 25 days without any access to food and can complete their development. Larvae and pupae placed in a controlled environment chamber at temperatures ranging between 20 and 22°C and a photoperiod of 12 h D: 12 h N failed to develop. This is an indication that an increase in photoperiod alone is not sufficient to break diapause, but that an increase in temperature or a combination of increasing day length, temperature and humidity may be needed to break larval diapause in *Tetramesa* sp. The laboratory data largely supports what was found in the field.

Various life stages of the wasp were found in the field throughout the study period with larvae generally being far more abundant in autumn and winter than in summer (Fig. 4). In contrast, pupae were found more often in culms in summer with the exception of February 2007. This anomaly may be as a result of the fact that January and February in 2007 were exceptionally dry months, compared to February 2006, in which a fair number of pupae were found in culms. The presence or absence of emergence holes is not significant as it could not be determined if the adults had emerged in years prior to or during the survey. According to Lees (1955) the termination of diapause is controlled primarily by a specific stimulus such as temperature, day length, or moisture. In a study done by Schneiderman and Horwitz (1958) on two parasitic chalcid wasps, *Mormoniella vitripennis* (Walker) and *Tritneptis Klugii* (Ratzeburg), it was found that diapause occurred in the last larval stage, the same as in *Tetramesa* sp. Exposing these parasitic wasps to low temperatures induced diapause and increasing the temperatures to 25ºC terminated diapause. However, many insect species have a strategy of bet-hedging to spread the risk of experiencing unfavourable conditions (Hopper, 1999) which is why all life stages are generally present at any one time during the year. A polymodal emergence strategy reduces the chances of localized extinctions after unseasonal climatic events. It is suggested that adult *Tetramesa* sp. emerge from culms a couple of days after the first rains when grasses have started sprouting new shoots and there are numerous oviposition sites available. The presence of rapidly dividing, nutrient-rich plant cells within the developing culm and inflorescence allow the larvae to develop fairly rapidly. The presence of a range of culms at

various stages of development means that there are suitable oviposition sites available throughout the summer.

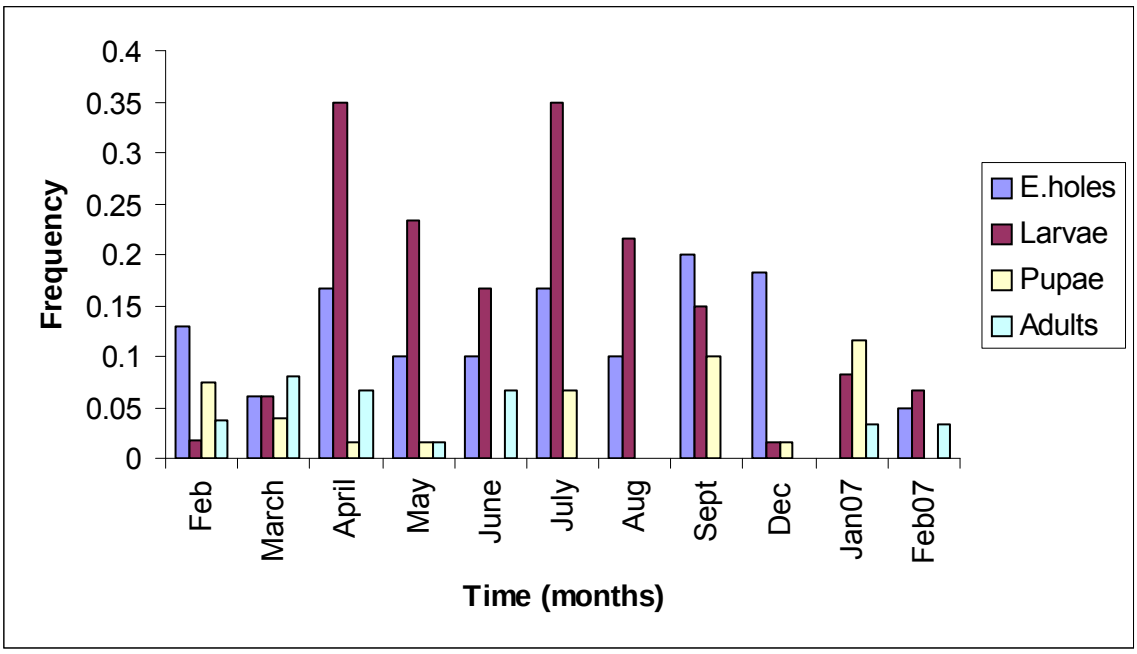

Figure 4: The seasonal occurrence of *Tetramesa* sp. emergence holes (E. holes), larvae, pupae and pre-adults found in the culms of *Sporobolus pyramidalis* over a period of 12 months in 2006/2007 (n=60 per month).

From laboratory studies it would appear that *Tetramesa* sp. probably has multiple generations per year, being able to complete its life-cycle in a relatively short period of time. Multivoltine insects tend to be small like *Tetramesa* sp. and develop faster than univoltine insects (Gullan & Cranston, 1994; Brown, 1984). *Tetramesa* sp. larvae, collected in the field generally pupated in the laboratory within 2-3 weeks, with adults emerging 6-7 days later. A leaf-feeding eurytomid, *Eurytoma* sp., collected on *Bryophyllum delagoense* was able to complete its development within 30 days (Witt *et al*., 2004), indicating that some eurytomids have the ability to complete their development within a very short period of time and it is expected that *Tetramesa* sp. will be no different.

A large number of larvae removed from culms were not *Tetramesa* sp. but other eurytomid species. Unfortunately, species could not be distinguished from each other at the larval stage; all larvae were white to cream in colour and looked similar in external morphology. However, adult *Tetramesa* sp. could be easily distinguished from other eurytomid species by its distinctive golden “shoulders” and elongated petiole and abdomen. In almost all cases approximately 30% of the larvae collected were *Tetramesa* sp., the others were mainly eurytomid species but it is unknown if they were phytophagous or parasitic. It is not unusual to find more than one eurytomid species in a particular grass species.

The fact that so few of the eurytomid larvae and pupae were *Tetramesa* sp. made it all that more difficult to establish a laboratory culture. In addition, larvae and pupae collected in the field were at

varying stages of development which meant that they did not all emerge as adults at the same time. Adults were also not particularly long-lived which meant that a large number of adults could not be placed on potted grasses, in cages, at any one time. Although we managed to place at least 100 adults on potted grasses throughout the year we never had more than five adults present in a cage at any one time.

Impact

Culm and inflorescence lengths for each month were positively correlated with longer culms generally always associated with long inflorescences. There was no apparent difference in this relationship between infested and healthy culms and inflorescences as shown for February (Fig. 5). However, it should be noted that far fewer infested than uninfested culms were found in the field, which may bring the results of this analysis into question. The results obtained by Witt and McConnachie (2003) were also contradicted in this study in that infested culms were not significantly shorter than healthy culms (Fig. 6). Infested inflorescences were also not significantly shorter than healthy ones (Fig. 7).

However, it was found that culms with more emergence holes, larvae, pupae and pre-adults were significantly shorter than those with fewer life stages (Fig. 8). This indicates that larval feeding does have an impact on culm length and that the comparative analyses between infested and healthy culms should be viewed with circumspection because there were only 11 infested culms used in the analyses that had more than five emergence holes, pupae, pre-adults or larvae.

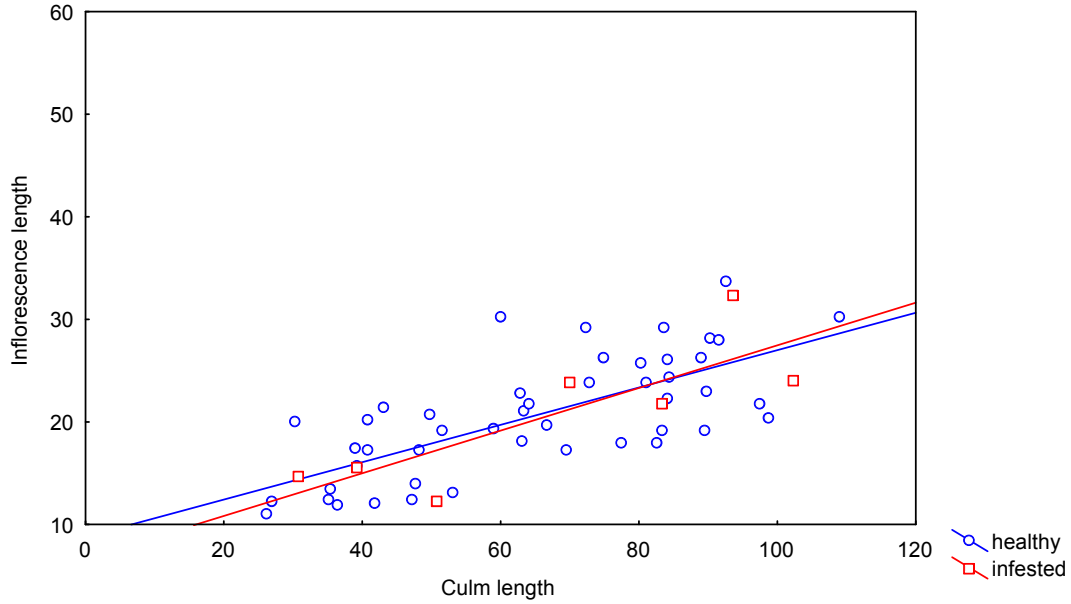

Figure 5: The effect of *Tetramesa* sp. infestation on the inflorescence length of *Sporobolus pyramidalis* for the month of February (Healthy: $R^2 = 0.5110$; P = 0.00000002; Infested: $R^2 = 0.6792$; P = 0.0226).

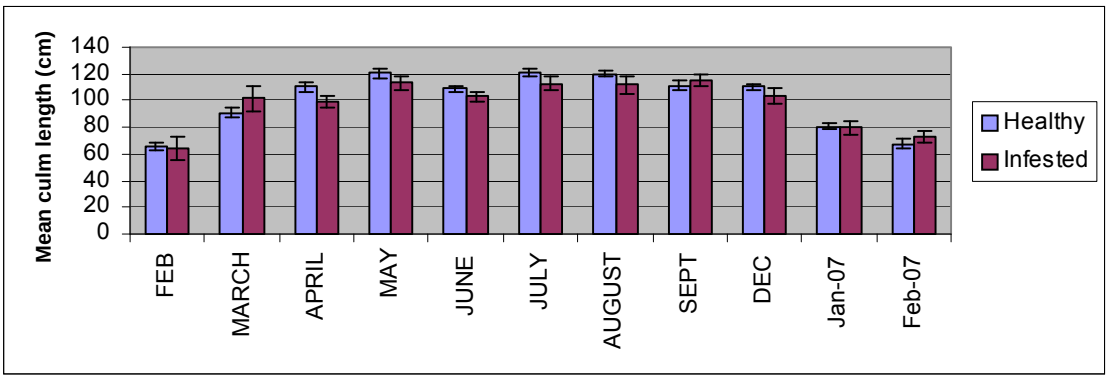

Figure 6: The mean culm length (cm) of healthy and infested *Sporobolus pyramidalis* grass culms over a period of 12 months in 2006/2007 (R^2 = 0.9011; ANOVA: F = 82.041; P = 0.422; t = 1.782288).

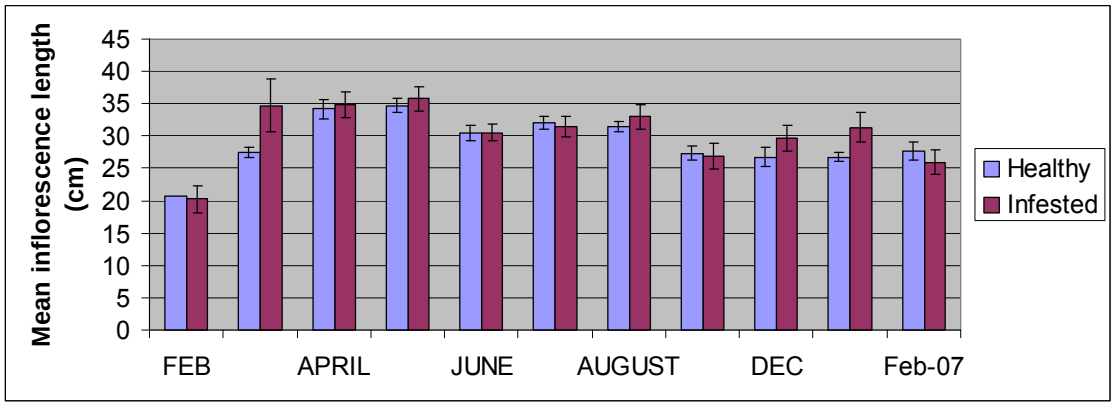

Figure 7: The mean inflorescence length (cm) of healthy and infested *Sporobolus pyramidalis* grass culms over a period of 12 months in 2006/2007. (R^2 = 0.844511; ANOVA: F = 27.15662; P = 0.319227; t = 1.795885).

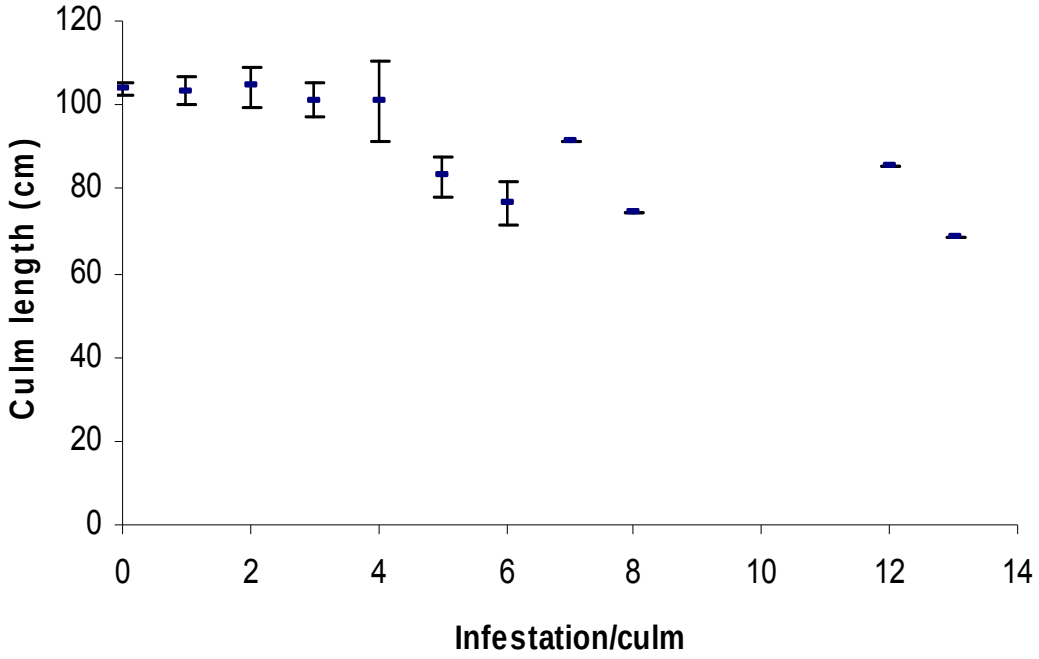

Figure 8: The impact of increasing numbers of *Tetramesa* sp. life stages, including emergence holes, on mean culm length of *Sporobolus pyramidalis* .

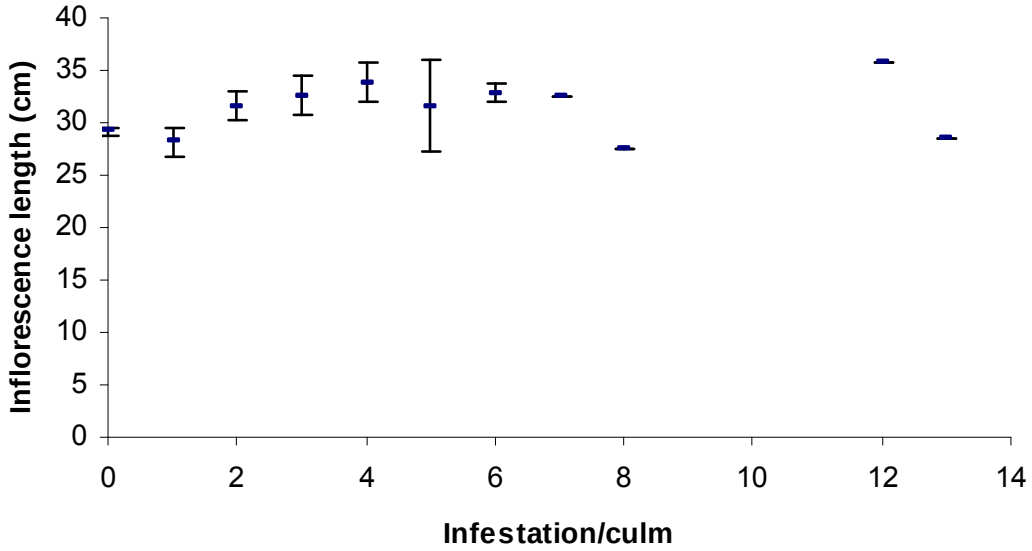

Figure 9: The impact of increasing numbers of *Tetramesa* sp. life stages, including emergence holes, on mean inflorescence length of *Sporobolus pyramidalis*.

CONCLUSIONS

Unlike the results of the preliminary study it was found that *Tetramesa* sp. had a negligible impact on culm and inflorescence length. However, further analyses of the data indicated that high numbers of larvae in culms (more than five) did have an impact on culm but not inflorescence length. Unfortunately, it could not be determined if larval feeding in culms had an impact on seed weight or viability.

Unfortunately, it was not possible to establish a *Tetramesa* sp. laboratory culture due to a number of factors, the main one being that insufficient numbers of larvae were collected in the field. In addition, adults were very short-lived which meant that to few adults could be exposed to potential oviposition sites at any one time.

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9.4 Appendix 4: Comments on *Ustilago sporoboli-indici*

Summary of comments from reviewers asked to comment on whether the results obtained in the first phase of testing of *Ustilago sporoboli-indici* justified further research.

Reviewer	Comment
Dr Jane Campbell Director Science and Natural Resources Dept. of Environment & Water Resources	I have had a number of conversations with people in the Department and there is general agreement that it is unlikely that approval would be given to the import of any agent that was likely to have an adverse impact on a native species. You are, however, free to apply for an amendment to be made to the live import list so that an assessment can be carried out.
Dr Shane Campbell Professional Leader Department of Natural Resources and Water Tropical Weeds Research Centre, Charters Towers	I agree with all the previous feedback circulated on this matter. We have to be cautious with progressing with this agent given its potential to infect native sporobolus species. The receipt of feedback from external stakeholders would be useful for this one, given that, as you say, many of the native sporobolus species although fairly widespread in native grasslands usually only make up a small proportion of the pasture composition and are not considered all that favourably compared with other species present. Nevertheless it is the issue about potential damage to a native species that will be the main impediment.
Dr Kunjithapatham Dhileepan Senior Entomologist Alan Fletcher Research Station Invasive Plant & Animal Science Biosecurity Queensland Department of Primary Industries & Fisheries	My comments are purely on scientific grounds. The results (physiological host range) clearly indicate that the agent is not specific enough to consider for release. In the current risk aversion climate, a lot more data are required even to consider the agent for risk analysis. One option is to conduct choice trials in the glasshouse and field (in South Africa) with various sporobolus species under different inoculum levels. This could provide some information on the inoculum levels at which it pose a thread to native sporobolus species. If this level is less than the level required to have any impact on the target weed, then it is not worth progressing further. If is more, then a risk analysis, as we have done for *Charidotis* (Raghu *et al*., Ecol. Modelling, in press) could be carried our. Even to consider for *Biocontrol Act* this information would be required.
Michael Day Entomologist Alan Fletcher Research Station Department of Primary Industries & Fisheries	There are several considerations which have been touched on such as specificity and effectiveness. These two issues suggest that it would be prudent to address both before proceeding. If we were to go down the Biocontrol Act, it might be worth waiting to see how other agents fare due to the cost and timeliness. With regard to effectiveness of the smut, as Dane Panetta highlights, I think putting a lot of effort in trying to get an agent that may not be effective approved for release may be unwise. I'm not sure it will come out well in a cost/benefit analysis if it may not control/damage the weed.

David Officer Pastures Research Officer Grafton Agricultural Research and Advisory Station NSW Dept. Primary Industry Grafton	1) Without appropriate testing *on Sporobolus virginicus* there is no chance that *Ustilago sporoboli-indici* will be approved for introduction into Australia. If testing cannot be done in Africa then it will need to happen here in Australia before release can be contemplated. 2) Further testing on the integration of herbicides and *Ustilago sporoboli-indici* looks interesting and necessary given the lack of effect on plant biomass and flowering for the full range of species determined to be susceptible. Discovery of a means of increasing *Ustilago sporoboli-indici* pathogenicity (eg successful herbicide and *Ustilago sporoboli-indici* integration experiments) is necessary for progression of this potential biocontrol agent. Ideally *Ustilago sporoboli-indici* would only affect weedy sporobolus and none of the natives. We have not been that lucky. However, the low pathogenicity of *Ustilago sporoboli-indici* could be a positive thing if human intervention can significantly improve its pathogenicity. For example *Ustilago sporoboli-indici* could be released where it is needed and with management (it will need to be cheap and capable of being applied to large areas safely) will reduce weedy sporobolus infestation densities to low levels. In this scenario *Ustilago sporoboli-indici* will have minimum impact on weedy sporobolus and for that matter all other sporobolus in the field without human input. This should be seen as a positive by those concerned about potential off target damage from *Ustilago sporoboli-indici*. 3) My comments on the distribution of natives and weedy sporobolus need to be verified with survey work if introduction on *Ustilago sporoboli-indici* is to be contemplated. This can be done with the appropriate funding and expertise. In my experience weedy sporobolus species are often found with little or no native sporobolus present (NSW North coast). I have seen a small percentage of infestations often newer or sparse infestations on native and naturalised grassland were both the weedy and native species are found together. In the latter case there is almost always more weedy sporobolus than native. In my view all three requirements must be met if *Ustilago sporoboli-indici* is to have a future as a classical biocontrol agent.
Dr Wayne Vogler Weed Scientist Tropical weeds Research Centre Biosecurity Queensland	My initial thoughts are that the road ahead will be difficult, how difficult will depend on the approving agencies and whether the Biocontrol Act needs to be engaged. While the native sporobolus are not significant to the pastoral or dairy industries it is too simplistic to say they are ecologically insignificant and therefore are of little consequence and can be lost without little impact. I doubt anyone really knows how significant they are as they really have not been studied very much. I think the idea that they are insignificant is based largely on their abundance or lack of in grasslands etc rather than any objective study and therefore caution is needed.

	If the climatic range of the rust were known it could be argued that it may only affect the native species in certain areas while leaving significant areas of these native species untouched which may give some hope to the approval process. I think if this project was to proceed some very careful modelling of the likely introduced range of the rust and an analysis of the native sporobolus within and outside this range and the likely overall effect on a national basis to native species would need to be conducted. This would help with the decision making process by giving some objective information as to the likely impact on native sporobolus nationally. After reading most of the discussion, some of the report and to answer Bill's question, I think we should cut our losses now and discontinue the project.
Dr Louise Morin CSIRO Entomology CRC for Australian Weed Management	I have not had time to read the report but my first feeling is that you would need to have evidence that the smut would have a major impact on the targeted exotic weed for regulators to accept possible 'lateral' damages on non-target native species. Do you have any data on epidemiology and impact of the smut in South Africa?
Dr Richard G. Silcock Principal Scientist (Pasture Agronomy), Department Primary Industries & Fisheries, Queensland	It does seem like you have little room to move for a number of reasons. My observations are these, 1. *S. elongatus* & *S. creber* are, as Bryan Simon said, of no obvious economic value and are usually a minor component of a pasture where many other perennial grasses co-exist. *S. elongatus* can become more abundant in traprock pastures west of Stanthorpe that are heavily utilised. Both species are easy to germinate but seedling vigour is poor so few survive under normal pasture conditions. 2. The lack of tests against *S. mitchellii* and *S. australasicus* pose problems from my perspective. *S. mitchellii* is an important river bank and floodplain stabiliser in inland Qld, so nothing should be brought in without clearing it of susceptibility. *S. australasicus* is a pioneer species in tropical Australia that probably plays an important role in land stabilisation where other things have been lost. 3. I would also think that *Thellungia advena* would have to be included because it is very like those true rat's-tail sporobolus species. It also has low forage or ecological value but once the possibility of genetic shift is included in the mix, where to then?

	We pasture gurus take some flack about what we should have known about bringing in exotic plants but I feel the same can be said of any lifeform.
Dr Dane Panetta Principal Scientist /Professional Leader Invasive Plant and Animal Science Biosecurity Queensland Department of Primary Industries and Fisheries	I was particularly interested in the following passage from the report: The smut fungus is slow to cause infections. In most cases, infections are not uniformly spread out in all treatments. For the smut fungus to be considered as a possible classical biological control agent, there is a need for more work to be done on how to improve the performance of the fungus. Trials using very low doses of 2,4-D and Round-Up to increase the susceptibility of the alien species to the smut are needed. Sublethal doses of herbicide suppress or inhibit the biosynthesis of compounds such as phytoalexins, enzymes or hormones, making the plant more susceptible to infection by pathogens. Herbicides such as glyphosate and 2,4-D have been used in a weed/pathogen system to enhance susceptibility of weeds, hence improving the efficacy of the classical biological control agent (Charudattan, 1986; Sharon *et al.,* 1992). We believe that this approach is technically and economically feasible, and would like to include it in future trials. The suggestion here is that, for whatever reason(s), *Ustilago* may not be a goer as a classical biocontrol agent. If there is a need to spray sublethal doses of herbicides to enhance its effectiveness, then perhaps infestations should be sprayed with lethal doses and be done with it. I believe that if there is a need for any additional inputs to get this agent to work well, then the benefits potentially arising from classical biocontrol will be significantly diminished. This is an issue beyond that of non-target attack, which I believe will be of far greater concern.
Dr Gabrielle Vivian-Smith Principal Scientist, Invasive Plants & Animal Science Biosecurity Queensland Department of Primary Industries and Fisheries	My initial thoughts are that we really need to seek some progress with the *Biocontrol Act* before moving forward (even cautiously) with this species, given it is likely to fall into the mother-of-millions situation. If possible, I would prefer to see the issue discussed at a reference group or workshop level with some MLA representation and possibly other external input (eg. via Weeds CRC).
Bryan Simon Principal Botanist Queensland Herbarium EPA, Brisbane Botanic Gardens	I would not be very concerned about biocontrol agents attacking these *S. elongatus* and *S. creber*, as they are also both a bit weedy. As far as I know they do not have much other ecological value. However I agree with the comments of Richard Silcock regarding *S. australasicus, S. mitchellii* and *Thellungia advena.*

Dr Roger Shivas Curator Plant Pathology Herbarium (BRIP) Plant Science Department of Primary Industries and Fisheries	This work needs to be completed to get a clearer picture. The photographs seem to suggest that they are good hosts - I don't think one can argue that it is a glasshouse artefact. The distribution in Australia of hosts needs to be compared to the targets. Also their phylogenetic relationship to each other and whether smut infection reflects this should be considered. It would be still be useful to see if *Eragrostis* spp. or other close relatives of *Sporobolus* are infected. Critical also is the degree of disease development - do these infected hosts produce fertile seed?
Dr Kálmán Vánky Herbarium Ustilaginales Vánky (HUV) Gabriel-Biel-Str. 5 D-72076 Tübingen Germany	I do not have any good idea what could be done in such a situation. Maybe to abandon the whole idea???
Dr Sangita Shrestha, Ph.D. Nepal Academy of Science and Technology	Not only the genetic diversity of host species but diversity of the pathogen is also important in causing any disease. Effective disease develops when a virulent race meets the susceptible variety but not when the pathogen is avirulent or the plant is resistant. You might have taken this into account in your research. Insights on inter and intra-specific genetic diversity (preferably at population genetic level) of host plays crucial role in determining host specificity of a number of diseases and pests of agricultural importance. With regards to weedy *Sporobolus* species of Australia, 11 pecies have been assigned of being members of '*S. indicus* complex'. Therefore, these are genetically closely related species. In phylogenetic terminology, they are monophyletic. However, we also have to remember that the five weedy species of Australia are introduced species and their centre of origin and global distributions are different. Hence during adaptation in various environments in Australia, they might have further acquired genetic changes through mutations and hybridizations. Our ITS sequence based phylogeny has revealed five major clades in the cladograms. One of the clades comprises of four species viz. *S. creber, S. elongates, S. sessilis* and *S. laxus.* The second clade comprised of *S. pyramidalis, S. natalensis* and *S. acquemontii* (although *S. jacquemontii* has further diverged into a sub-clade). The third clade comprised of *S. fertilis* and *S. fricanus*. *Sporobolus indicus* and *S. diandrus* formed the fourth and fifth clades. Therefore, it would be better if other species of '*S. indicus* complex' were also included in the study. According to your result, one of the endemic species, *S. cabridus* is also susceptible to smut fungus indicating that the host range

	of smut fungus may still be much broader than suspected. Therefore, it would have been better if you had considered more endemic species as well. As in cases of true species, population level genetic diversities can also make differences with regards to host specificity of various diseases and pests, although actual results are all empirical. Much closer genetic relationship among *S. laxus, S. sessilis, S. longatus* and *S. creber* group revealed from our RAPD study has been further substantiated from our ITS sequence based phylogenetic study. Therefore, these four species are more loosely related to each other than to other remaining species of the complex. Similarly, in *S. pyramidalis, S. natalensis* and *S. jacquemontii* cluster, GRTGs are more closely related to each other than to ARTG. Therefore, this may be the reason for the difference in result you obtained for host specificity with *S. jacquemontii.* Genetic diversity studies at population level could provide important clues regarding polymorphic behaviour of the host species under consideration and hence the probability of spread of any particular disease. Chances of survival of host from the disease epidemics are higher in polymorphic species than in species with less genetic diversity. Our RAPD study also provided some clues regarding intra-specific genetic diversity of the weedy *Sporobolus* species. However, this study not being a population level study, the results of polymorphisms of various species could not be absolutely relied upon. From our RAPD study, *S. natalensis* was found to be the most polymorphic species followed by *S. fertilis, S. elongtatus* and *S. pyramidalis.* Finally, classical biological control can be one of the best options to stop spread of these weeds. However, Integrated Pest Management (IPM) strategy should be followed in order to achieve the goal of weedy *Sporobolus* spp. management in Australia and every possible solutions have to be employed (including molecular diagnostic) in order to maintain pasture productivity in Australia.