MEAT & LIVESTOCK AUSTRALIA

On farm

Biocontrol of Sporobolus Grasses

African survey for weedy sporobolus biocontrol agents

Project number NBP.304

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ISBN 1 74036 508 9

March 2004

Natural Resources

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Abstract

Five grasses (*Sporobolus africanus (Parramatta grass), S. fertilis (Giant Parramatta grass), S. jacquemontii (American rats tail grass) and S. natalensis* and *S. pyramidalis (both Giant rats tail grass*), 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 usually uneconomic, biological control offers a practicable solution.

The Queensland Department of Natural Resources and Mines, 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.

Over two years, areas infested with these grasses were surveyed from Western Cape Province in the south to northern Botswana, resulting in the collection of over 70 phytophagous insect species and 23 plant pathogens.

Two organisms were selected as being potential biocontrol agents and recommended for further study. The leaf smut, *Ustilago sporoboli-indici* attacked all three grass species and was very damaging. Spores of the smut could be germinated and clean plants infected in the laboratory. A eurytomid wasp, *Tetramesa* sp., was found infecting the grasses and causing malformed inflorescences. However, it was not cultured in the laboratory.

Executive Summary

Five grasses (*Sporobolus africanus (Parramatta grass), S. fertilis (Giant Parramatta grass), S. jacquemontii (American rats tail grass) and S. natalensis* and *S. pyramidalis (both Giant rats tail grass*), 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 usually uneconomic, biological control offers a practicable solution.

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The study was undertaken over a two year period, 2001-2003, from the South Africa Field Station situated near Pretoria, South Africa. The full time staff of the South African Field Station; the Senior Researcher Arne Witt and the Senior Technician Andrew McConnachie primarily undertook the survey. Both these people are entomologists by training. A plant pathologist, Dr. Isabel Rong, who also identified most of the pathogens, joined them on some trips. In December 2002 Dr. Roger Shivas, Senior Plant Pathologist Queensland Department of Primary Industry, and Dr. Kalman Vánky, a smut specialist, spent a month in South Africa searching for further pathogens. Identifications of the plant specimens were made by Dr. Lynne Fish of the National Botanic Institute ARC and Dr. Roger Ellis, Group Head ARC Plant Genetic Resources Unit, advised on the distribution of the grasses. Insect identifications were made by staff of the National Collection of Insects.

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 issues. 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* presented some difficulty, as they are morphologically quite similar. Further, these species interbreed. The taxonomy of the weedy sporobolus grasses remains problematic and is outside the scope of this project. By project's end the survey team was confident in their diagnoses but also collected appropriate plant specimens at collecting sites so that future changes in species concepts can be accommodated. *S. 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 and many specimens have not yet been identified. Many of the species will only be partially determined (usually to genus) as they belong to groups that have not yet been properly described in southern Africa. Most of these species represent casual association with the plant rather than utilizing the grass as a true host plant.

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. All attempts to rear this insect in the laboratory were unsuccessful. Up to four other undescribed eurytomid wasp species, some possibly parasitic, were also found in the stems and this issue also remains to be resolved.

Twenty-three pathogens, including five primary pathogens, were found on the *Sporobolus* spp. Only the leaf smut *Ustilago sporoboli-indici* was thought to be 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. Dr. Vánky was also able to use his data from the South African trip to complete his paper describing all the smut fungi known from *Sporobolus.* This paper has now been published and will be a valuable resource document.

The project will generate several publications. Grasses have been largely eschewed as targets for biological control for a number of reasons and this is one of the first attempts to find agents for a problematic grass. Publications will therefore make a valuable contribution the science of biological control of weeds.

Introduction

Five grasses (*Sporobolus africanus (Parramatta grass), S. fertilis (Giant Parramatta grass), S. jacquemontii (American rats tail grass) and S. natalensis* and *S. pyramidalis (both Giant rats tail grass*), collectively known as the weedy sporobolus grasses, are serious pastoral weeds in Australia, affecting productivity, property management and, ultimately, land values. 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.

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. A typically successful biocontrol might return a benefit/cost ratio of $2-10 per research dollar and in some cases this is considerably higher.

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.

Weedy grasses have only recently been targeted for biological control. 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 in their natural enemies), and the great adaptability of grasses to grazing and harvesting (see Appendix A for a full discussion).

The weedy sporobolus grasses are all exotic and belong to a section of the *Sporobolus* genus known as the *indicus* complex. 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 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.

Because 3 of the 5 weedy species originate in southern Africa, this area was 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.

The purpose of the study was to survey southern Africa over two growing seasons for all the invertebrate fauna and pathogens associated with the three species. After having all collected organisms identified by expert taxonomists, any potentially promising potential biocontrol could then be selected and studies of their biologies and host specificity commenced.

Methods

The Survey Area

Surveys were carried out over most of the known distribution of the three *Sporobolus* spp. in South Africa, Swaziland, Botswana and Namibia. Twenty-eight trips were undertaken during the 18 month survey period. One hundred and eighty-eight different field sites were visited on 234 occasions. Unfortunately relatively few sites were found in Swaziland and Botswana despite intensive surveys in these countries. Only a handful of sites were found alongside the main roads in Swaziland and most of these sites had very few insects probably as a result of the relatively dry summer in 2002/2003. A drought, compounded by heavy overgrazing in the

southern and eastern parts of Botswana, made it difficult to find any good *Sporobolus* sites. However, the northern regions had received good rains and *S. pyramidalis* was relatively common at some sites along the Chobe River. Two *Sporobolus* sites in northern Namibia (Kavango and Caprivi) were also surveyed.

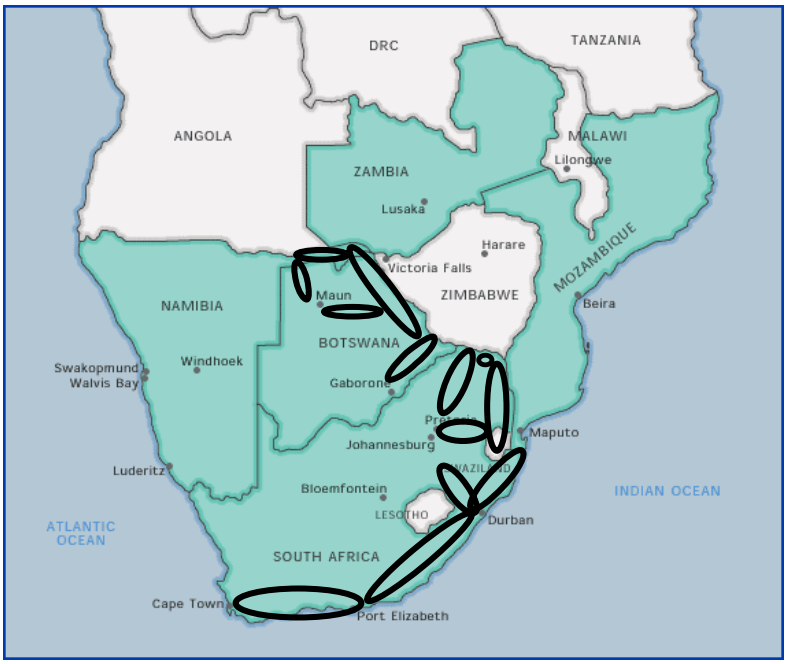

Fig. 1. Areas surveyed for arthropods and pathogens on *Sporobolus* spp.

Unlike the situation for Botswana and Swaziland, most of the *Sporobolus* sites in South Africa were surveyed on more than one occasion. Due to their close proximity, most localities in and around Pretoria were surveyed on more than two occasions while some sites near Nylstroom in Limpopo Province were surveyed at least five times. Many of the sites in the Eastern and Western Cape Provinces were visited at least twice.

Identification of the Grasses

All three *Sporobolus* spp. were found in abundance in southern Africa. Unfortunately, the three species in the *S. indicus* complex are known to hybridise amongst themselves and with *S. fimbriatus*, making identification very difficult even for experienced grass taxonomists such as Dr. Lynne Fish. Many specimens sent for identification had characteristics of two *Sporobolus* species. However, with the large number of specimens being brought in for identification, Dr. Fish was able to use other characteristics to distinguish between the species with relative confidence. The proportion of sites with each species were as follows:

S. pyramidalis – 42%; *S. africanus* – 43%; and *S. natalensis* – 15%. The relative proportion of time devoted to surveying each species (in terms of the number of sites visits) was: *S. pyramidalis* – 45%; *S. africanus* – 44%; and *S. natalensis* – 11%.

Collection of Specimens

Due to the taxonomic impediment every effort was made to collect at least one grass specimen from every locality surveyed. A flowering plant was removed with its roots intact, placed in a plant press and appropriately labelled. In the laboratory all specimens are mounted, labelled and sent to the National Herbarium at the National Botanical Institute in Pretoria for identification by Dr. Fish.

Various methods can be used to collect insects on plants. In these surveys, most insects and/or other organisms were located visually, captured using a pooter (aspirator) and placed in a killing jar containing ethyl acetate. Detailed information on the organisms and aspects of the site were recorded on a collection sheet. At the end of each day insects were pinned and placed together with provisional labels. Other insects, such as scales and aphids, which are less mobile and in many cases sessile, were collected by removing the plant part on which they were feeding and placing in alcohol. Immature insects were collected and placed in larger containers with some foliage. These were reared in the laboratory and killed and pinned when they reached the adult stage. All organisms collected and reared were accurately labelled, giving the exact locality, date, collector/s and species of plant on which they were collected and then sent to the National Collection of Insects in Pretoria for identification by specialist taxonomists.

Plants infected with pathogens were collected in the field by removing the infected part of the plant, labelling and placing in a cooler box. Upon return to the laboratory they were furnished with more comprehensive labels and sent to the Plant Pathology section of the ARC-PPRI for identification by Dr. Isabel Rong. Dr. Roger Shivas, Senior Plant Pathologist QDPI, and Dr. Kalman Vánky, the smut fungi expert from Germany, spent the whole of December 2002 with SAFS staff in the field in South Africa looking for primary pathogens. They also looked over all previously collected material (see Appendix B for details of this trip).

Results

Pathogens

Twenty-three pathogens were isolated from *Sporobolus* spp. (Table 1) and at least partially identified. Five primary pathogens were found. One of these is a promising agent for the control of invasive *Sporobolus* spp. while the other four are already present in Australia. The remaining organisms were secondary pathogens and with no potential as biological control agents.

The five identified primary pathogens were a leaf rust (*Uromyces tenuicutis* McAlp.), tar spot (*Phyllachora sylvatica* Sacc. & Speg.), choke disease (*Parepichloë cinerea* Berk. & Br.), ear blight (*Bipolaris crustacea* (Henn.) Alcorn) and a smut (*Ustilago sporoboli-indici* L. Ling. *Ustilago sporoboli-indici* is a potential agent but the other four organisms are already present in Australia.

The smut, *U. sporoboli-indici* appears to be a most promising pathogen. The smut produces sori on the leaves and culms and usually prevents the production of an inflorescence. The disease appears to be systemic and usually all shoots of an infected tiller are sterile. In surveys where 10 randomly collected *S. pyramidalis* plants at each of five localities were separated into individual tillers, only 6% (15/250) of infested tillers had inflorescences compared to 50% (547/1085) of uninfected tillers. The culms of infested tillers were also significantly shorter than uninfected tillers [74.6cm (n=15) and 101.8cm (n=547); df = 14,

t=3.46; p < 0.002]. A transect survey at five localities revealed that on average 54% (range = 15-70%) of grass clumps had at least one infested tiller.

The smut is widespread and was found on *S. pyramidalis*, *S. natalensis*, and *S. africanus* throughout the regions surveyed in southern Africa. It is also known to occur in other parts of Africa, Asia and the Philippines (Vánky 2003 and Appendix C) but has never been recorded in Australia. Its wide distribution and ability to infect a large number of shoots and cause infected plants to become sterile indicates that it has excellent potential as a classical biocontrol agent in Australia.

Bipolaris crustacea, which infects the reproductive parts of the grass was widespread throughout the survey area and was particularly abundant on *S. africanus* in the Western Cape Province, with up to 99% of inflorescences being heavily infected at some sites. However, it is already in Australia, where trials have found it to be ineffective because of low rates of infection and the timing of infection in relation to seed production (Hetherington, 1997). The other primary pathogens are also already in Australia, where they appear to have a negligible impact on *Sporobolus* spp.

Table 1. Pathogens collected and identified on *Sporobolus* spp. in southern Africa

Species	**Notes**
Alternaria alternata (Fr. : Fr.) Keissler	
Bipolaris crustacea (Henn.) Alcorn	On inflorescences of *Sporobolus africanus, S. capensis,*
Bipolaris hawaiiensis (M.B. Ellis) Uchida & Aragaki	On many different grasses and other plants i.e.
Cladosporium sp.	Known leaf pathogens, some species host specific
Curvularia lunata(Wakker) Boedjin,	Plurivorous, causes leaf spots and seedling blights.
Curvularia sp.	Pathogens of many grasses
Dactylaria sp.	
Fusarium sp.	Common on seeds of specimens submitted
Hochapfel sp.	
Myrocthecium sp.	Saprotrophic, prominent on roots of specimens submitted
Nigrospora sphaerica (Sacc.) Mason	Prominent on young leaflets of specimens submitted
Parepichloë cinerea Berk. & Br.	Choke disease
Periconia byssoides Pers: Mérat	Common on dead plant material
Periconia sp.	Saprotrophic, prominent on roots of specimens submitted
Pithomyces sp.	Prominent on seeds of specimens submitted
Phoma glomeratta (Corda)	Common on leaves of specimens submitted
Phoma sp.	Prominent all parts of the plant submitted
Phyllachora sylvatica Sacc. & Speg.	Tar spot
Ramichloridium schulzeri (Sacc.) de Hoog	A saprotroph, common on wheat straw.
Uromyces tenuicutis McAlp.	Leaf rust
Ustilago sporoboli-indici L. Ling	Damaging
Verticillium sp.	Saprotrophic, prominent on roots of specimens submitted
Wollenweber sp.	

Dr. Vánky conducted preliminary experiments on *U. Sporoboli-indici* on his return to Germany, using material collected in South Africa. Firstly he demonstrated that the spores collected from both *S. africanus* and *S. pyramidalis* germinated extremely well (90-100%) and that it did not matter whether spores had been frozen. Secondly, he demonstrated that seedlings could be infected by dropping a suspension of germinated spores onto 1-6 day old plants and that it did not matter whether the spores were taken from the same host species. This experiment also indicated that infection by the smut could be very damaging to young plants. Details are provided in Appendix D.

Insects

More than 70 phytophagous arthropods were collected on *S. pyramidalis*, *S. africanus* and *S. natalensis* and at least partially identified (Table 2). The most abundant and widespread species collected included: *Stramia* pr. *costirostris* (Coleoptera: Curculionidae), *Micraspis comma* (Coccinellidae), *Monolepta cruciata* (Coleoptera: Chrysomelidae), *Afroeurydemus* sp. (Coleoptera: Chrysomelidae), *Horridipamera nietneri* (Lygaeidae), *Eysarcoris inconspicuus* (Pentatomidae), *Balclutha rosea* (Cicadellidae), *Exitianus taeniaticeps* (Cicadellidae), *Haplothrips stofbergi* (Phlaeothripidae) and *Tetramesa* sp. (Eurytomidae).

Fewer arthropods were collected in the second year than the first because of the dry summer in 2002/2003. Although most of the arthropods collected in the summer of 2002/2003 have not yet been identified, there did not appear to be any damaging arthropods collected this summer that were not collected in 2001/2002. The most promising arthropod agent, the stem-boring wasp, *Tetramesa* sp. was found at many additional sites and despite the dry summer was abundant at most of these. The wasp was collected from *S. pyramidalis*, *S. africanus* and *S. fimbriatus*.

Field surveys have indicated that infestations are relatively high at most localities. Of the 144 *S. pyramidalis* culms randomly collected at one site, 33% were infested with *Tetramesa* sp. larvae. The inflorescences of 60% of these infested culms were malformed. The infested culms were also significantly shorter than uninfested ones, with lengths of 470 mm and 656 mm respectively. In an attempt to determine if *Tetramesa* sp. is having an impact on the weight of seeds *Sporobolus* spp., culms were randomly collected from the field. The length and diameter of culms were recorded, as was the number and position of emergence holes, larvae and pupae in each culm. Seeds were removed from each culm and placed in a petri-dish. All seeds were then placed in a drying oven and 25 seeds from each culm were then weighed. Using regression analysis, no correlations were found between the number of *Tetramesa* sp. life stages present and inflorescence length ($R^2 = 0.0501$), culm length ($R^2 = 0.0002$) or seed weight ($R^2 = 0.0014$). Seed weights from grasses infested with *Tetramesa* sp. and uninfested grasses were also found not to differ significantly ($df = 54$; $t = -0.12$; $p = 0.45$).

All attempts to establish a *Tetramesa* sp. laboratory culture over an 18 month period were unsuccessful and were hampered by high levels of parasitism. Over and above the two undescribed *Tetramesa* sp., three additional undescribed eurytomid species have been reared from *Sporobolus* sp. culms and it is not known if these are parasitic or phytophagous.

Table 2. Phytophagous insects associated with *S. pyramidalis, S. natalensis* or *S. africanus.*

Order	Family	Subfamily/Tribe	Species
Coleoptera	Curculionidae	Myorhinini	*Umzila capeneri* Marshall
		Cyphicerini	*Myllocerus* nr. *auriceps* Fåhraeus
		Cyclomini	*Stramia* pr. *costirostris* (Boheman)
		Oosomini	*Glyptosomus* sp.
		Peritelini	*Lalagetes* sp.
	Tenebrionidae	Lagriinae	*Lagria* spp.
	Meloidae		*Decapotoma lunata* Pallas
	Scarabaeidae	Hopliini	2 spp.
	Cerambycidae	Cerambycinae	*Ossibia fuscata* (Chevrolat)
	Chrysomelidae	Galerucinae	*Monolepta cruciata* Guérin-Méneville
			Monolepta sp.n.
			Monolepta bioculata (Fabricius)
			Monolepta congener (Jacoby)
			Medythia nigricollis (Bryant)
			Afrosoma cf. *suturale* (Allard)
			Afromaculepta frontalis (Chevrolat)
			Asbecesta cyanipennis Harold
			Altica cuprea Jacoby
		Alticinae	*Phygasia* sp.
			Chaetocnema sp.
			Smaragdina terminata (Lacordaire)
		Clytrinae	cf. *Coptocephala* sp.
			Cryptocephalus cf. *bistripustulatus* Suffrian
		Cryptocephalinae	*Macrocoma* cf. *aureovillosa* (Marshall)
		Eumolpinae	*Afroeurydemus* spp. (2)
Hemiptera	Aphididae		*Hysteroneura setariae* (Thomas)
	Margarodidae	Monophlebinae	Unknown spp.
	Miridae		Unknown spp.
	Lygaeidae		*Horridipamera nietneri* (Dohrn)
			Horridipamera inconspicuus (Dallas)
			Atrademus papeneri (Slater)
			Nysius pallidus Evans
			Nysius natalensis Evans
			Paromius gracilis (Rambur)
			Spilstethus pandurus elegans (Wolff)
	Pentatomidae		*Eysarcoris inconspicuus* Herrich-Schaeffer
			Durmia headula (Stal)
			Menidia transversa (Signoret)
			Aspavia albidomaculata Stal
			Bolbocoris inaequalis Germar
			Aeliomorpha caffrae (Westwood)
			Bolbocoris rufus Germar
			Eysarcoris inconspicuus Herrich-Scaeffer
	Cercopidae		*Locris* sp.

Table 2. Phytophagous insects associated with *S. pyramidalis, S. natalensis* or *S. africanus*

Order	Family	Subfamily/Tribe	Species
			Locris sanguinipes Walker
			Locris aenea Distant
			Locris arithmetica Walker
	Ricaniidae		*Mulvia albizona* Germar
	Cixiidae		*Pentastridius moestus* (Stal)
	Meemoplidae		Genus and species unknown
	Cicadellidae		*Balclutha rosea* (Scott)
			Exitianus taeniaticeps Kirchbaum
			Austroagallia sp.
			Tetartostylus sp.
			Glossocratrus afzelii (Stal)
			Hecalus sp.
			Recilia aulonias Linnavuori
			Empoascanara ethiopica Dworakowska
			Typhlocybinae spp. (3)
			Recilia sp.
			Exitianus pr. *capicola* (Stal)
			Balclutha sp.
	Trophiduchidae		*Numica viridus* Muir
	Flatidae		*Gyaria walkeri* Stal
	Scutelleridae		*Deroplax nigropunctata* (Stal)
	Coreidae		*Clavigralla elongata* Signoret
Thysanoptera	Phlaeothripidae		*Haplothrips stofbergi* Faure
Hymenoptera	Eurytomidae		*Eurytoma* spp. [5 spp]
			pr.*Eurytoma* spp. [3 spp]
			Bruchophagus sp.
			pr. *Bruchohagus* sp.
			Tetramesa spp [2 spp]
Diptera	Chloropidae		*Thaumatomyia natalensis* (Becker)
			Pachylophus proximus Adams
Orthoptera	Acrididae	Acridinae	*Acrida* sp.
	Tettigoniidae		*Conocephalus* sp.
Lepidoptera	Lymantriidae		*Lacipa gemmata* Distant
	Tortricidae		*Panemus robusta* (Walker)
	Notodontidae		*Antheura ornata* (Walker)

Discussion

Surveying the grasses over a two year time frame allowed for a thorough study of the invertebrate fauna and pathogens. Although several areas of southern Africa were “off limits”, it was still possible to search over much of southern Africa and to observe many areas at different times of the year and in different years. As anticipated, many organisms could not be fully identified because they belonged to groups that have not yet been fully studied. Although the full time staff were specialist entomologists, the search for pathogens was supported by several plant pathologists, including some with international reputations. For these reasons, it is felt that the undertaken survey was thorough.

Two organisms, *Tetramesa* sp. and *Ustilago sporoboli-indici,* have been identified as potentially good biocontrol agents in this project. They will both need detailed study and host specificity testing if they are to be progressed as biocontrol agents. A very high degree of host specificity will be required because there are closely related native congeners present in Australia. The second requirement is that there are good prospects that the agent will be damaging.

The leaf smut, *Ustilago sporoboli-indici* is clearly the most promising agent. It was seen to be very damaging in both the field and the laboratory. Further it has not yet been recorded on any plant species outside the species comprising the weedy sporobolus grasses and the very closely related *S. indicus.* Preliminary experiments have also indicated that its spores can be germinated in the laboratory and that uninfected plants can be experimentally infected. These are essential first steps to studying its biology and host specificity.

A smut, *Sporisorium ophiuri,* is already being considered for the control of itch grass, *Rottboellia cochinchinensis,* in Costa Rica. It is extremely damaging and as a sole agent, based on the results of a model, could reduce the population of itch grass by 90% over 20 seasons, with an annual infection rate of 85% (Smith *et al*., 1997). This level of infection is unlikely to be achieved consistently but nevertheless indicates how damaging this agent can be. Infected plants also have significantly fewer tillers and leaves, and also flower earlier than healthy individuals.

The host specificity of biotrophic pathogens can be extremely narrow, sometimes being restricted to a particular biotype as demonstrated with the rust, *Puccinia chondrillina* released for the control of skeleton weed in Australia (Burdon *et al*., 1981). Smuts can also be extremely host specific (Valverde *et al.*, 1999).

The second prospective agent, *Tetramesa* sp., is also quite promising.

Spears and Barr (1985) found that *Tetramesa* spp. reduced seed weight in *Aristida longiseta*, *Sitanion hystrix*, *Sporobolus cryptandrus* and *Stipa comata* 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). *Eragrostis teff* 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). This is therefore a clear indication that stem-boring eurytomids can have a significant impact on seed production.

Many phytophagous eurytomid species are also monophagous. Martinez *et al*. (1999) found 18 species of phytophagous eurytomids in 10 sympatric species of grass, with no species occurring in more than one species of grass. Ten grass species from Germany supported 15 *Tetramesa* spp. and one *Eurytoma* spp. (Tscharntke *et al*., 2001). Of these 16 species, 12 were monophagous, 1 had three grass species as hosts and the host ranges of the remaining 3 were unknown (Tscharntke *et al*., 2001). The *Tetramesa* sp. collected on *Sporobolus* spp. in southern Africa should also have a limited host range and may not attack any Australian native *Sporobolus* spp.

Before host testing can proceed, further information on the biology of *Tetramesa* sp. needs to be acquired to facilitate the establishment of a laboratory culture. Great care will need to be taken to separate emerging *Tetramesa* sp. adults from field material from other eurytomids possibly present in order to eliminate parasitism.

Some comment should be made about the risks associated with pursuing this research to the stage of receiving approval to release the two agents in Australia.

The major risk is that the two nominated agents will not be sufficiently host specific to be approved for release in Australia. The biological control of grasses is a relatively new undertaking and there has yet to be an approval for release of an agent for a grass species. This lack of corporate experience, together with the very great importance that grasses occupy in respect to both economic and ecological aspects, means that those approving release are likely to be conservative in outlook and will require very thorough host testing to be undertaken. Additionally we know that there are some 26 native *Sporobolus* sp. present in

Australia, including some placed in the *indicus* group. Some of the native *Sporobolus* spp. have important ecological function, while others are described as rare and endangered. The specificity of the two nominated agents will need to be very narrow; most probably confined to the five weedy sporobolus grasses that are the target for the research.

It is quite likely that either or both agents might be rejected because their host range is too broad. It would be important to know whether the agents might attack the closely related species very early in the project so that the agent is only subjected to the full host testing process (which may well involve a large approved test list) if it does not attack a small selected group in preliminary tests. Preliminary testing can be undertaken with a reasonably small budget and this approach is proposed in the research application.

The second risk relates to the possibility of getting the weedy sporobolus grasses approved as targets for biological control (*query – shouldn't this actually come first? – why start the research if approval is not going to be forthcoming?*). Approval for a weed to be a target for biological control is usually given by the Natural Resource Management Standing Committee (NRMSC) on the advice of the Australian Weeds Committee (AWC). Recently some members of the AWC have indicated a very conservative approach to this matter and have attempted to direct any weed with any even a minimal degree of contention through the Biological Control Act (BCA), which provides legal indemnity (*query – has this been established with certainty?*). The BCA has only rarely been used. Even though NRMSC has recently supported the use of the BCA, it might well take 2-3 years to get approval as a target by this route. In the case of the weedy sporobolus grasses it is known that some graziers in northern NSW regard Giant Parramatta grass as a useful back-up drought fodder reserve and for this reason it was thought that AWC may recommend that the BCA be used. However a verbal agreement has been reached that the proposal to have the weedy sporobolus grasses declared as targets for biological control will be put jointly by Queensland, NSW and Victoria. Under this arrangement there would have to be a high expectation that the proposal will be approved without undue problems.

A third concern involves the eurytomid wasp, *Tetramesa* sp. Although this insect has been found to be quite abundant on *Sporobolus* sp. and has been regularly collected from field samples, it has still not been reared through a generation in the laboratory. This is an essential early step in the research on this insect. Because other species of Eurytomidae are associated with this plant, it will be necessary to work from a purified laboratory culture for all the host specificity trials. Further it is highly desirable to have a laboratory culture to provide material for import into Australia. Resolution of this problem will be attempted by contracting appropriate scientists in Pretoria to tackle this aspect first. If it is not possible to culture *Tetramesa* in the laboratory, further work on this agent would be stopped.

In summary, there are risks associated with the continuation of this project and it is by no means a certainty that both or even one agent will be brought to the stage of safe release in Australia. However the problematic areas of research can and have been identified and can therefore be addressed at an early stage of the project and before the bulk of the research budget need be invested. It also must be stated that the benefits, should this project be successfully completed, will be of at least one order of magnitude greater than total research expenditure and that there is therefore an opportunity to bring about a major benefit to the meat producing stakeholder.

Acknowledgements

The staff of the South African Field Station Arne Witt, Andrew McConnachie and Sharon Dougherty undertook the majority of the field survey, analysis of data and reporting of findings. Dr. Kalman Vanky, Dr. Roger Shivas and Dr. Isabel Rong contributed to the project as specialist plant pathologists. Staff at the National Insect Collection, particularly Riaan Stalls and Beth Groebellar, provided identifications of the collected insects. In Australia valuable advice was provided by Roger Shivas, Dane Panetta, Peter Mackey and Allan Tomley. Very strong industry support for this project was provided by the Weedy Sporobolus Management Group and its chair, Margaret Emmery.

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Appendix A

Paper presented to XI International Symposium on Biological Control of Weeds. Canberra. May 2003.

The potential for classical biological control of invasive grass species with special reference to invasive *Sporobolus* spp. (Poaceae) in Australia

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Running headline: Potential biocontrol of *Sporobolus* and other grasses

Summary.

Sporobolus africanus, *S. natalensis* and *S. pyramidalis* were accidentally introduced to Australia from Africa and have the potential to invade approximately 223 million hectares.

Mechanical and chemical controls are largely ineffective and expensive, hence the search for potential biological control agents in southern Africa. Mycoherbicides are being used more widely today for the control of some invasive grass species in agricultural situations although no pathogen has been released as a classical biocontrol agent.

Arthropods have been largely ignored as potential agents until very recently because it was assumed that the simple architecture of grasses and the lack of secondary compounds would militate against the evolution of monophagy. However, in recent surveys of *Phragmites australis* and *Calamagrostis epigejos* in Europe some monophagous insect species have been found, and *Prokelisia marginata* (Delphacidae) has been released for the control of *Spartina alternifolia* on the west coast of the United States. Many *Tetramesa* spp. (Eurytomidae) are apparently monophagous and a species that has been reared from *S. pyramidalis* in South Africa is extremely damaging.

A number of other damaging insects have been collected on these *Sporobolus* spp. but can only be considered as potential agents once they have undergone further trials.

Many pathogens have also been collected including a leaf rust (*Uromyces tenuicutis*), but a smut (*Ustilago sporoboli-indici*) appears to have the most potential. The biggest obstacle to the biological control of invasive *Sporobolus* spp. in Australia is the fact that there are 13 native *Sporobolus* spp., which will largely govern which agents can be selected for biocontrol.

This paper considers the various factors which make grasses amenable to biological control and criteria used in the selection of agents, with particular reference to invasive *Sporobolus* species in Australia.

Keywords: Grasses, pathogens, rust, smut, *Sporobolus*

Introduction

Grasses cover more of the world's land surface than any other vegetation type. Grasses are the most important food crops in the world and are also utilized extensively for building materials, essential oils, ornamental plants, lawns and pastures. As a result grass species have been introduced, either accidentally or intentionally, to many regions worldwide.

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. All of the introduced species are unpalatable to livestock and the carrying capacity of invaded pastures can be reduced by 10-80% resulting in a potential loss of A$60 million per annum to the livestock industry in northern Australia (Dept. of Natural Resources & Mines 2001). It has been estimated that this complex of invasive species could invade approximately 223 million hectares (Dept. of Natural Resources & Mines 2001). Chemical and mechanical control measures have proved to be either ineffective, impractical or expensive, hence the search for potential biological control agents in southern Africa.

A number of potential agents have been found in surveys of *S. africanus*, *S. pyramidalis* and *S. natalensis* in South Africa, Swaziland and Botswana. In this paper we report on progress towards the selection of control agents for this complex of *Sporobolus* spp. and comment more broadly on the selection of grasses as targets for biological control.

Sporobolus spp. taxonomy and biology

There are approximately 160 *Sporobolus* spp. in tropical and subtropical areas (Clayton and Renvoize 1986). Of the 21 *Sporobolus* species in Australasia, 13 are endemic (Simon and Jacobs 1999). However, the recognition of many of these species, especially those in the *S. indicus* complex, is difficult because of the morphological intergradation in the genus (Simon and Jacobs 1999). *Sporobolus pyramidalis*, *S. africanus* and *S. natalensis* are all known to hybridise making field identification very difficult (Van Wyk and Van Oudtshoorn 1999).

Species in the *S. indicus* complex occur on all soil types and generally in areas with high rainfall (Van Wyk and van Oudtshoorn, 1999). *Sporobolus pyramidalis* occurs throughout tropical Africa as well as Madagascar, Mauritius and Yemen while *S. africanus* and *S. natalensis* are found from southern Africa to East Africa as far north as Ethiopia (Van Wyk and van Oudtshoorn, 1999). Weedy *Sporobolus* spp. can mature in as little as three months under favourable conditions (Dept. 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 (Dept. of Natural Resources & Mines 2001).

Grasses as targets for biological control

According to Randall (2002), 18146 plant species have become invasive worldwide. Of these, 13670 are dicotyledons, and 4476 are monocotyledons, of which 2176 are species in the family Poaceae. The family with the greatest number of invasive species is the Asteraceae followed by the Poaceae and Fabaceae (Table 1) (Randall, 2002). The top five species of weed worldwide, based primarily on the impact they have in agriculture in control costs and yield reduction (Holms *et al*. 1977), are in the Cyperaceae or Poaceae, with *Cyperus rotundus* L. being the worst weed worldwide (Holm *et al*. 1977).

To date, species in 40 plant families have been selected as targets for biological control (Julien and Griffiths 1998). Most are in the families Asteraceae (31 spp.), Cactaceae (23 spp.), Fabaceae (Mimosoideae, Caesalpinioideae, Papilionoideae) (19 spp.) and Rosaceae (4 spp.) (Julien and Griffiths 1998). Control programmes have never been initiated against any species in the Poaceae and only two species in the Cyperaceae have had agents released for their control despite the abundance of weedy species in these two families. This is possibly because grasses are perceived as lacking specific herbivores, and as being too similar in morphology, physiology and ecology to crop species (Gill and Blacklow 1984; Evans 1991). The apparent absence of host specific arthropods has been ascribed to their simple structure and lack of secondary compounds, which reduces the evolution of monophagy (Evans 1991). This view was entrenched by surveys on *Imperata cylindrica* and *Cyperus rotundus* in the early 1970s (Simmonds 1972) and *Sorghum halepense* in Northern Italy in the 1980s (Domenichini *et al*. 1989) which found that arthropods on these species were not sufficiently host specific and/or damaging. As a result, arthropods were widely discounted as potential control agents for grasses, with most attention focussing on the use of mycoherbicides (Evans 1991).

However, recent evidence would appear to suggest that even simple plants like grasses support large numbers of arthropods. A recent literature survey by Tewksbury *et al*. (2002). found more than 160 arthropod species associated with *Phragmites australis* (Cav.) Trin ex Steud. *Spartina alternifolia* Lois. has more than 24 arthropod species which have potential as biological control agents (F.S. Grevstad, University of Washington, *pers. comm*.) while *Calamagrostis epigejos* (L.) has10 endophagous arthropod species (Dubbert *et al*. 1998). In any case, the number of species associated with a plant should not necessarily deter from its selection as a target species. Many simple plants like *Opuntia* spp. and water weeds have been successfully controlled despite the fact that they have few arthropod species associated with them in their native ranges (Moran 1980; Julien and Griffiths 1998).

The fact that alkaloids are only present in less than 0.2% of grasses while other noxious terpenoids and chemical compounds are completely absent (McNaughton *et al*. 1985) should also not deter from their selection as target species. Recent evidence suggests that the role of plant toxicity in fostering monophagy has been overemphasized and that other explanations may be preferable (Futuyma and Keese 1992). Structural defences like trichomes, silica bodies and others may also play a role in driving monophagy in insects (Djamin and Pathak 1967).

Weed species with no closely related native species or crops are seen as better targets than weeds with native congeners (Pemberton 2000). Oligophagous species like *Cactoblastis cactorum* (Bergroth) and *Dactylopius opuntiae* (Cockerell) could be released against *Opuntia* spp. in South Africa because there are no native species in the Cactaceae and no closely related major crop species (Moran 1980). The family with the most species targeted for biological control, the Asteraceae (Julien and Griffiths 1998), contains no major crop species other than sunflower (Simmonds 1976). In contrast, the Poaceae which has no species targeted for biocontrol, has the highest percentage of weedy species and has more than 20 species of major crops, more than any other family (Simmonds 1976). Nevertheless, weed species have been selected as targets despite being closely related to major crops (Julien and Griffiths 1998). *Solanum elaeagnifolium* was selected as a target weed in South Africa despite there being many major crops in the same genus (Olckers *et al*. 1999). However, agents released for the control of invasive *Sporobolus* spp. in Australia will need to be extremely host specific to appease environmentalists because there are 13 (62%) endemic *Sporobolus* spp. in Australasia and two of these species are listed as rare and one as vulnerable in Queensland (Simon and Jacobs 1999).

Introduced invasive grass species may also be overlooked as biocontrol targets because they are not noticed in native grasslands, especially if they have many native congeners, and their impact is therefore seen as being negligible. Until the public can distinguish between

native and introduced grasses and is made aware of the impact they have on native ecosystems, grasses will continue to be ignored unless a problem in agricultural situations.

Selection of biological control agents for grasses

Both pathogens and arthropods were considered as potential biological control agents for weedy *Sporobolus* spp. A number of criteria were used to select potential biological control agents: impact on the host plant, host specificity, distribution, and ease of rearing. The most important initial consideration was impact on the target plant and subsequently the host specificity of the agent. Agents which could reduce seed production were considered to be the best option at this early stage.

According to Moran (1980) the arthropod complex on simple plants should be dominated by endophagous species eg. *Opuntia* spp. where 79% of the phytophagous species are borers (Lepidoptera and Coleoptera) (Moran 1980). Grasses, being simple plants, should therefore also be dominated by endophages. However, according to Tscharntke and Greiler (1995) grasses are dominated by ectophages, which is what we found on *Sporobolus* spp. in our surveys. However, in *P. australis*, there are virtually an equal number of ectophages and endophages (Tewksbury *et al*. 2002), probably because the large culms provide niches for a large number of arthropods. Endophagous species are also abundant in other large semi-aquatic grasses like *S. alternifolia* and *C. epigejos*.

Unlike the situation in many dicotyledons where the arthropod fauna is often dominated by species in the Coleoptera (Curculionidae and Chrysomelidae) (Syrett *et al*. 1996) grasses have a relatively poor beetle fauna (Tewksbury *et al*. 2002). Only eight beetle species have been collected on *P. australis* worldwide (Tewksbury *et al*. 2002). However, in smaller grasses, like *Sporobolus* spp. and *N. trichotoma,* beetles are relatively abundant but the majority of these are generalist pollen feeders. Diptera (Agromyzidae, Chloropidae) are generally more common in grasses than in dicotyledons, with 32 species in the Chloropidae, most of them endophagous, collected on *P. australis* (Tewksbury *et al*. 2002). Herbivores with apparent specialization on *S. alternifolia* are mainly hemipterans with only two of the 24 arthropod species being coleopterans (Mordellidae, Curculionidae) (F.S. Grevstad, University of Washington, *pers. comm*.).

Host specificity of agents on grasses

Chewing insects on grasses are generally oligophagous (Bernays and Berbehenn 1987), but many other taxa are monophagous. There is a close association between many species in the Cecidomyidae and particular grass hosts (Barnes 1946) and many grass-feeding homopterans also have a small host range (Southwood and Leston 1959; Gibson 1976). Many stem-boring and stem-galling dipterans found in grasses have a limited host range (Nye, 1959; Mowat, 1974), with more than 20 monophagous chloropid species attacking *P. australis* (Tewksbury et al. 2002). Other families with a large number of monophagous species on *P. australis* are the Agromyzidae and Delphacidae while species in the Pseudococcidae, Coccidae and Noctuidae are generally polyphagous (Tewksbury et al. 2002). Of the nine endophagous insects collected on C. epigejos two are considered to be monophagous (Eurytomidae, Chloropidae) (Dubbert et al. 1998).

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. The position in which the larvae develop on the culm is also specific for many species (Bouček 1988) as demonstrated by the endophages on *C. epigejos* (Dubbert *et al*. 1998).

Many pathogens on grasses also only have a single host with head smuts and many rusts

being extremely host specific (Valverde *et al*. 1999). The host specificity of biotrophic pathogens in general can be extremely narrow, sometimes being restricted to a particular biotype as demonstrated with the rust *Puccinia chondrillina* released for the control of skeleton weed in Australia (Burdon *et al*. 1981). A pathogen that exhibits biotype selectivity within a single species should not infect plants from closely related species.

Level of damage caused by agents on grasses

Arthropods on grasses can be extremely damaging and result in the death of the attacked plant. A sap-sucker, *Prokelesia marginata* (Van Duzee) (Homoptera: Delphacidae), recently released for the control of *S. alternifolia* on the west coast of the United States, was placed in cages with *S. alterniflora* plants from Willapa Bay (Daehler and Strong, 1997) and *S. anglica* plants from Puget Sound (Wu *et al*., 1999). Attacked plants from both species were severely stunted or died.

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). 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. and 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).

A stem-borer *Tetramesa* sp. (Hymenoptera: Eurytomidae), collected on *S. pyramidalis*, *S. africanus* and *S. natalensis* in southern Africa was also found to be damaging. Of 144 *S. pyramidalis* culms randomly collected at a particular site 33% were infested with *Tetramesa* sp. larvae. The inflorescences of 60% of these infested culms were malformed. The culms of infested plants were also significantly shorter {470mm (n = 48) vs 656mm (n = 96); df = 79, t = -6.385, $P < 0.001$)}.

Numerous pathogens damage cereal crops throughout the world, with smuts and rusts being particularly abundant. A smut, *Sporisorium ophiuri,* which is being considered for the control of *Rottboellia cochinchinensis* in Costa Rica, is very damaging and as a sole agent could reduce the density of itchgrass by 90%, with an annual infection rate of about 88% (Smith *et al*. 1997). This level of infection is unlikely to be achieved consistently but indicates how damaging a smut can be. Infected plants have significantly fewer tillers and leaves and flower earlier than healthy individuals.

Of the five primary pathogens collected on the three *Sporobolus* spp. the smut, *Ustilago sporoboli-indici* L. Ling appears to be the most promising agent. The other pathogens, a leaf rust, (*Uromyces tenuicutis* McAlp.), tar spot (*Phyllachora sylvatica* Sacc. & Speg.), choke disease (*Parepichloë cinerea* Berk. & Br.) and, ear blight (*Bipolaris crustacea* (Henn.) Alcorn) are all ready present in Australia (R. Shivas, Queensland Department of Primary Industries, *pers. comm*.) while the smut has only ever been recorded in other parts of Africa, Asia and the Philippines (Vánky, *in prep*.). Research into the use of *B. crustacea* as a mycoherbicide found that it was not suitable anyway because of its low rates of infection and the timing of infection in relation to seed production (Hetherington and Irwin 1999).

Ustilago sporoboli-indici produces sori on the leaves and stems and usually prevents the production of an inflorescence. The disease appears to be systemic and usually all shoots of an infected plant are affected and sterile. In preliminary surveys, 10 randomly collected *S. pyramidalis* plants at each of five localities were separated into individual tillers, and only 6% (15/250) of infested tillers had inflorescences compared to 50% (547/1085) of uninfested tillers. The culms of infested tillers were also significantly shorter than uninfested tillers

{74.6cm(n = 15) vs 101.8cm (n = 547); df = 14, t = 3.46, P < 0.002}. In transect surveys at five localities, an average of 54% (range = 15-70%) of grass clumps had at least one infested tiller.

Conclusions

There does not appear to be any valid reason why grasses should not be considered as targets for classical biological control programmes. Recent surveys on a number of grass species clearly demonstrate that there are large numbers of arthropods, especially on large species, and that many of them are monophagous. We are optimistic that some of the agents we have selected as potential biocontrol agents for *Sporobolus* spp. will be both damaging and host specific.

Acknowledgements

Thanks to Drs. R. Shivas and K. Vanky for assistance in field surveys, and to Drs. W.A. Palmer, R. McFadyen, M.P. Hill, J.H. Hoffmann, C. Moran and colleagues in ARC-PPRI for valuable comments on the manuscript.

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Appendix B

SURVEY FOR PATHOGENS OF

SPOROBOLUS IN SOUTH AFRICA,

4-29th DECEMBER 2002

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Summary

Specimens of diseased *Sporobolus* grasses belonging to the complex comprising *S. africanus*, *S. fimbriatus*, *S. natalensis* and *S. pyramidalis* were collected across South Africa in December 2002. Five major pathogens were found. Leaf rust (*Uromyces tenuicutis*), and tar spot (*Phyllachora sylvatica*) were widespread minor leaf pathogens. Choke disease (*Epichloë cinerea*), which destroys the inflorescence of infected plants, was found on *S. africanus* in Western Cape Province and on an unidentified species of *Sporobolus* in Mpumalanga Province. Ear blight (*Bipolaris crustacea*), which infects the ovaries of diseased plants, was only found on the previous season's inflorescences of *S. africanus* in Western Cape Province. The smut (*Ustilago sporoboli-indici*), was a widespread and severely damaging pathogen of these four species of *Sporobolus* at most sites surveyed in South Africa. The sori of the smut appear on the leaves and stems and render infected plants sterile, which indicate that it has excellent potential as a classical biocontrol agent in Australia.

Introduction

The South African *Sporobolus* grasses in the complex comprising *S. africanus* (Poir.) Robyns & Tournay, *S. fimbriatus* (Trin.) Nees, *S. natalensis* (Steud.) Dur. & Schinz and *S. pyramidalis* Beauv., are becoming increasingly important weeds that adversely affect agricultural and environmental areas in eastern Australia (Natural Resources and Mines, 2001). There are no biological controls currently available to manage these grasses (McFadyen, 1999). Since 2001, DNR&M has been searching for potential biocontrol agents for weedy *Sporobolus* in southern Africa (Palmer, 2002). As part of this project, a survey specifically for plant pathogens of weedy *Sporobolus* was carried out in December 2002.

Methods

Specimens of diseased *Sporobolus* were collected from more than 50 locations across South Africa in December 2002. Herbarium specimens (dried and pressed) were prepared from the collected material and forwarded to QDPI Plant Pathology Herbarium (BRIP) under Australian Quarantine and Inspection Service permit no. 200111199, which stipulates the heat treatment of these specimens to render the pathogens non-viable. Specimens of smut (*Ustilago sporoboli-indici*) were deposited in Herbarium Ustilaginales Vánky (HUV). Duplicates of all of the collected specimens will be forwarded to Dr Isabel Rong, South African Mycological Collection, Pretoria (PREM).

The itinerary for the survey follows.

6 December 2002	PREM, Pretoria
7 December 2002	National Botanical Gardens, Pretoria
8 December 2002	Nylstroom, Northern Province
9 December 2002	PREM, Pretoria
10 December 2002	Bergville, KwaZulu - Natal
11 December 2002	Kokstad, KwaZulu - Natal
12 December 2002	Mkambati, Eastern Cape Province
13 December 2002	Port St. Johns, Eastern Cape Province
14 December 2002	Cintsa, Eastern Cape Province
15 December 2002	Grahamstown, Eastern Cape Province
16 December 2002	George, Western Cape Province
17 December 2002	Graaf-Reinert, Western Cape Province

18 December 2002	Pretoria
19 December 2002	National Institute of Botany, Pretoria
20 December 2002	Songivelo Game Reserve, Mpumalanga
21 December 2002	Mkuze, KwaZulu-Natal
22 December 2002	Vryheid,KwaZulu-Natal
23 December 2002	Pretoria
24 December 2002	Dullstroom, Mpumalanga
25 December 2002	Sabie, Mpumalanga
26 December 2002	Steenkampsberg, Mpumalanga
27 December 2002	Pretoria
28 December 2002	Mhlabatini Kloof, Mpumalanga

Results and Discussion

More than 100 specimens of diseased *Sporobolus* were collected and retained as reference material. Five diseases and their pathogens were identified, namely leaf rust, (*Uromyces tenuicutis* McAlp.), tar spot (*Phyllachora sylvatica* Sacc. & Speg.), choke disease (*Epichloë cinerea* Berk. & Br.), ear blight (*Bipolaris crustacea* (Henn.) Alcorn) and smut (*Ustilago sporoboli-indici* L. Ling).

Leaf rust and tar spot were widespread, minor leaf pathogens. Both of these pathogens occur in Australia. Neither pathogen is considered to have potential as a biocontrol agent.

Choke disease caused by *Epichloë cinerea*, destroys the entire inflorescence, replacing it with an ascostroma. It was found on *S. africanus* in Western Cape Province and an unidentified species of *Sporobolus* in Mpumalanga Province. The pathogen is certainly more widespread as herbarium records at PREM showed that it also occurs on *S. pyramidalis* in Kwa-Zulu Natal Province. It also occurs in Australia.

Ear blight caused by *Bipolaris crustacea*, infects the ovaries of diseased plants. It produces a black, crustose fungal mass that often completely envelops the inflorescence (Alcorn, 1982). This pathogen was only found on the previous season's inflorescences of *S. africanus* in Western Cape Province. Although damaging, this fungus occurs in Australia. Hetherington (1997) does not consider it suitable for use as a mycoherbicide because of low rates of infection and the timing of infection in relation to seed production.

Smut (*Ustilago sporoboli-indici*) produces sori on the leaves and stems and usually prevents the production of an inflorescence. The disease appears to be systemic and usually all shoots of an infected plant are affected and sterile. It was a widespread and severely damaging pathogen of *Sporobolus* across the regions surveyed in South Africa. At one site in Mpumalanga Province, c. 70% of the shoots were infected. The pathogen is known to occur also in other parts of Africa, Asia and also in the Philippines (Vánky, 2003), but has never been recorded in Australia. The ability of this smut to infect a great number of shoots and cause infected plants to become sterile indicates that it has excellent potential as a classical biocontrol agent in Australia. Our observations are that the pathogen is restricted to *Sporobolus*. However nothing is known about the biology of *Ustilago sporoboli-indici*, including spore germination and the infection process.

Thoughts for the future

- The ability of *Ustilago sporoboli-indici* to attack young shoots, stunt plants and render infected plants sterile, makes it an excellent candidate for the biological control of weedy *Sporobolus* in Australia.

- The pathogens recorded on *Sporobolus* in Australia, as determined by the literature and herbaria records, may be incomplete. It would be prudent to complete a survey for pathogens of *Sporobolus* in Australia as well as to thoroughly examine herbarium specimens of diseased *Sporobolus* in Australia. Of particular interest are two unidentified specimens of smut on *Sporobolus* collected in southern Australia and held in Herbarium VPRI, Victoria.

- Smut fungi are a good source of potential biological control agents for grasses as many of them destroy inflorescences or render plants sterile thereby reducing seed production in infected populations. The smut fungus, *Sporisorium ophiuri*, has been studied by CABI (UK) as a classical biological control for *Rottboellia cochinchinensis* in Costa Rica. Some of this work may be relevant to the development of *Ustilago sporoboli-indici* as a biocontrol agent for *Sporobolus*.

- Little is known about the biology of *Ustilago sporoboli-indici*. Studies are needed to determine how spores germinate; how to culture it; how it infects plants as well as its host range. These studies could be undertaken at PPRI (South Africa), CABI (UK) and/or HUV (Germany).

- There may be pathogens of weedy *Sporobolus* outside of South Africa, as for example, other smuts are known to occur on *Sporobolus* (Vánky, 2003). In September 2003, KV is visiting Ethiopia and this could provide an opportunity to search there for pathogens, including smuts, on *Sporobolus*.

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Appendix C

Published as: Vánky, K. 2003. The smut fungi (Ustilaginomycetes) of *Sporobolus* (Poaceae). *Fungal Diversity* 14: 205-241.

The smut fungi (Ustilaginomycetes) of *Sporobolus* (*Poaceae*)

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New name: *Jamesdicksonia tremuli* Vánky (substituting *Melanotaenium sporoboli* Thirum. and M.C. Sriniv., type on *Sporobolus tremulus*, India). New combinations are: *Jamesdicksonia major* (Har. and Pat.) Vánky (based on *Entyloma majus*, type on *Sporobolus spicatus*, Chad); *J. sporoboli* (H.S. Jackson) Vánky (based on *Tolyposporella sporoboli*, type on *Sporobolus indicus*, Puerto Rico); *Macalpinomyces spermophorus* (Berk. and M.A. Curtis ex de Toni) Vánky (based on *Ustilago spermophora*, type on *Eragrostis poaeoides* USA); *M. spinulosus* (L. Ling) Vánky (based on *Ustilago spinulosa*, type on *Sporobolus paniculatus*, Sierra Leone); *M. sporoboli* (Tracy and Earle) Vánky (based on *Ustilago sporoboli*, type on *Sporobolus junceus*, USA); *Ustilago peruviana* (Zundel) Vánky (based on *Sphacelotheca peruviana*, type on *Sporobolus virginicus*, Peru); and *Ustilago utahensis* (Zundel) Vánky (based on *Sphacelotheca utahensis*, type on *Sporobolus patens*, USA). The host plant of *Ustilago schlechteri* Henn. is not a *Sporobolus* but an *Enneapogon* species. A neotype is designated for *Ustilago schlechteri*.

Key words: neotype, new combinations, new name, synonyms, taxonomy.

Introduction

Sporobolus R. Br., in the subfamily *Chloridoideae*, tribe *Eragrostideae*, subtribe *Sporobolinae*, has about 160 species in the tropics and subtropics (Clayton and Renvoize, 1986: 224). On *Sporobolus* at least 20 smut fungi have been recorded. Some of them are synonyms, others belong to genera other than that they were originally placed in, or the host plant is not a *Sporobolus*. For example, the host plant of the type of *Tilletia asperifolia* Ellis and Everh., *Sporobolus asperifolius* Nees and Mey. is now regarded as a synonym of *Muhlenbergia asperifolia* (Nees and Mey.) Parodi, and that of *T. montana* Ellis and Everh., *Sporobolus gracillimus* Vasey, is *Muhlenbergia filiformis* (Thurb.) Rydb. *Ustilago striiformis* (Westend.) Niessl has been collected in the USA on *Sporobolus auriculatus* Vasey, which is a synonym of *Muhlenbergia arenacea* (Buckl.) Hitchc. However, the specimens in BPI (167936, 167937) under this name, represent *Ustilago buchloës* Ellis and Tracy, on *Bouteloua* sp. *Ustilago schlechteri* Henn. was described on "*Sporobolus* sp.", which is *Enneapogon*, probably *E. scoparius* Stapf.

There is confusion regarding species delimitation and generic placement of many of the dark-spored "*Entyloma*" and "*Melanotaenium*" species of *Poaceae* and *Cyperaceae*, including those of *Sporobolus*. Since the work of Bauer *et al.* (1997), it is known that *Entyloma* and *Melanotaenium* species are restricted to dicotyledonous host plants only (comp. also Vánky, 2002). Bauer *et al.* (2001) demonstrated that "*Entyloma*" and "*Melanotaenium*" species of *Poaceae* and *Cyperaceae* belong either to *Jamesdicksonia* Thirum., Pavgi and Payak

(*Georgefischeriaceae*), to *Eballistra* R. Bauer, Begerow, A. Nagler and Oberw. (*Eballistraceae*), or to *Phragmotaenium* R. Bauer, Begerow, A. Nagler and Oberw. (*Tilletiariaceae*), all within the order *Georgefischeriales*. Knowledge of soral and spore morphology alone is insufficient to classify these fungi. For generic placement of these grass-infecting smuts, accurate knowledge of spore germination and/or molecular data is necessary. Unfortunately, these data are lacking for most of the "*Entyloma*" and "*Melanotaenium*" species of *Poaceae*. For now, it is best to place, by analogy, these fungi of *Sporobolus* into the genus *Jamesdicksonia.* However, caution! Similar, dark, blackish leaf spots on grasses may be produced also by ascomycetes. This is the case of "*Entyloma crastophilum* Sacc." on *Sporobolus asperifolius* Nees & Mey. (= *Muhlenbergia asperifolia*), USA, Utah, Salt Lake City, 18.VIII.1904, A.O. Garrett (BPI 175173!).

Several South African *Sporobolus* species in the complex comprising *S. africanus* (Poir.) Robyns and Tournay, *S. fimbriatus* (Trin.) Nees, *S. natalensis* (Steud.) Dur. and Schinz, and *S. pyramidalis* P. Beauv. are introduced, invasive weeds in Australia (Walton, 2001, Palmer, 2002). For their biological control, *Ustilago sporoboli-indici* L. Ling seems to be an excellent candidate.

Taxonomy

The sixteen recognised smut fungi on *Sporobolus* are:

1. *Jamesdicksonia major* (Har. and Pat.) Vánky, **comb. nov.** (Figs. 1A, 2-3)
Basionym: ≡ *Entyloma majus* Hariot and Patouillard, Bulletin du Muséum d'Histoire Naturelle (Paris) 15: 197 (1909). ≡ *Melanotaenium majus* (Har. and Pat.) Ciferri, Atti dell' Istituto Botanico dell' Università de Pavia, Ser. 3, 1: 95 (1924). **—** Type on *Sporobolus spicatus* (Vahl) Kunth, Chad, between Modou and Bérirem, ca. 110 km N of Fort Lamy, at southern fringe of Lake Chad, October 1903, A. Chevalier. (Holotype PC, isotypes BPI 175837, HUV 13671!).

Sori (Fig. 1A) forming lead-coloured, swollen, usually fusiform spots on the leaves, rarely also on the leaf sheaths, 0.5–1.5 x 1–4 mm, or larger by confluence, covered by the epidermis which later ruptures longitudinally, revealing the black, agglutinated spore mass embedded in the host tissue. The spore mass separates into single spores in water, under pressure.

Spores (Figs. 2, 3) variable in shape and size, globose, ellipsoidal, elongated or irregular, 8–14 x 10–16(–19) µm, medium to dark reddish-brown; wall two-layered, endospore even, ca. 0.5 µm thick, brown, exospore even or slightly uneven, from nearly non-existent up to 2.5(–3) µm thick, subhyaline to pale yellowish-brown, smooth, but may be finely wavy due to very low tubercles or ridges, evident especially in SEM.

Hosts: *Sporobolus cordofanus* (Steud.) Coss., *S. ioclados* (Trin.) Nees, *S. marginatus* Hochst. ex A. Rich. (*S. arabicus* Boiss.), *S. pyramidatus* (Lam.) Hitchc. (*S. argutus* (Nees) Kunth), *S. spicatus* (Vahl) Kunth.

Known distribution: Africa (Chad, Congo, Kenya, Sudan), Asia (Pakistan), West Indies (Dominican Rep.).

2. *Jamesdicksonia sporoboli* (H.S. Jackson) Vánky, **comb. nov.** (Figs. 1C, 4-5)

Basionym: ≡ *Tolyposporella sporoboli* H.S. Jackson, in Whetzel and Kern, Mycologia 18: 122 (1926).
≡ *Melanotaenium sporoboli* (H.S. Jackson) Thirumalachar, Whitehead and O'Brien, Mycologia 59: 394 (1967), (later homonym, not Thirum. and M.C. Sriniv., 1963/1964). **—** Type on *Sporobolus indicus* (L.) R. Br. (det. A. Chase), Puerto Rico, El Yunque, 14 April 1916, H.H. Whetzel and E.W. Olive 450. (Holotype BPI 178145!, isotypes BPI 178143 and 178144).

Sori (Fig. 1C) forming lead-coloured, slightly swollen, elongated spots or striae on the leaves, 0.3–1.5 x 1–7 mm, or longer by confluence, first covered by the epidermis which later ruptures longitudinally, revealing the black, agglutinated spore mass embedded in the host tissue. *Spores* (Figs. 4, 5) variable in shape and size, mostly rounded subpolyhedrally irregular, but also lacrymiform, lemon-shaped or elongated, more rarely globose or ovoid, 7–12 x 7–19 µm, pale olivaceous-brown; wall two-layered, endospore even, thin, ca. 0.5 µm, indistinct, exospore uneven, 1–6.5(–8) µm thick, with indistinct, concentric layers, smooth.

Hosts: *Sporobolus brockmanii* Stapf, *S. indicus* (L.) R. Br., *S. marginatus* Hochst. (comp. also Dennis, 1988).

Known distribution: Africa (Eritrea), Asia (Pakistan), West Indies (Puerto Rico).

3. *Jamesdicksonia tremuli* Vánky, **nom. nov.** (Figs. 1B, 6-7)
Substituting ≡ *Melanotaenium sporoboli* Thirum. and M.C. Sriniv., in Srinivasan and Thirumalachar, Sydowia 17: 22 (1963/1964), [not *Jamesdicksonia sporoboli* (H.S. Jackson) Vánky, opus praesens]. **—** Type on *Sporobolus tremulus* Kunth, India, Bombay State, Vadgaon, 11 July 1957.

Sori (Fig. 1B) forming lead-coloured, rounded, or ellipsoidal pustules on the leaves, 0.5–2 mm long, or longer by confluence, covered by the epidermis which later ruptures longitudinally, revealing the black, agglutinated spore mass embedded in the leaf tissue. *Spores* (Figs. 6, 7) agglutinated into irregular groups, single spores extremely variable in shape and size, usually irregular, with one or several flattened sides, often elongated, broadly subfusiform or also triangular, with one or several acute or subacute tips, 9–15 x 11–20(–28) µm, dark olivaceous-brown; wall two-layered, endospore even, thin, ca. 0.5 µm, exospore uneven, 1.5–7(–8) µm thick, smooth. *Spore germination* (Srinivasan and Thirumalachar, 1963/1964: 22) results in a holobasidium with 6–8 apical basidiospores.

Hosts: *Sporobolus diander* (Retz.) P. Beauv., *S. tremulus* Kunth, *S. wallichii* Munro.

Known distribution: Asia (India). I did not see the type of *J. tremuli*, but I have seen the collection on *Sporobolus wallichii* from India (HCIO 20563) which, according to Srinivasan and Thirumalachar (1963/1964: 22) "is identical with the species under study".

4. *Macalpinomyces spermophorus* (Berk. and M.A. Curtis ex de Toni) Vánky, **comb. nov.** (Figs. 8, 11-12)
Basionym: ≡ *Ustilago spermophora* Berkeley and M.A. Curtis ex de Toni, in Saccardo, Sylloge fungorum, etc. 7: 466 (1888). ≡ *Sphacelotheca spermophora* (Berk. and M.A. Curtis ex de Toni) Moesz, Botanikai Közlemények 19: 63 (1921). ≡ *Ustilago spermophora* Berk. and M.A. Curtis, in Curtis, Geological and Natural History Survey of North Carolina, Part 3, Botany: 123 (1867), (as '*spermophorus*'; nomen nudum). **—** Type on *Eragrostis poaeoides* P. Beauv. var. *megastachya* Koehler [= *Eragrostis cilianenis* (All.) Janchen], USA, Iowa, Charles City, September 1882, J.C. Arthur. (Isotypes in Ellis, N. Amer. fgi. no. 1098, as *Ustilago spermophorus*, HUV 10545!).

Further taxonomic synonyms are: = *Ustilago kusanoana* Henn.; = *U. orientalis* Yen; = *Sphacelotheca cheoana* Zundel (comp. Vánky, 1994: 376-377).

Sori (Fig. 8) in some ovaries of an inflorescence as 1–2 mm long, green, spherical or pyriform bodies between the glumes, covered by a peridium of fungal and host origin which ruptures irregularly to expose the dark brown, powdery spore mass intermixed with sterile cells, or they are apparently lacking. Sori usually fall off the plant. Often the distal part of the sorus bears a remnant of the caryopsis as a hard, yellowish-brown, acute body. Heavily infected panicles may be congested. *Spores* (Figs. 11, 12) globose, subglobose to ovoid, occasionally elongate or irregular, 6.5–9(–10) x 8–11(–13) µm, light brown, finely, moderately densely verrucose-echinulate; spore profile finely serrulate. *Sterile cells* (Fig. 11) globose, subglobose,

ellipsoidal, rarely elongate, 5.5–8 x 6–11 µm, hyaline, collapsed in old specimens; wall thin, ca. 0.5 µm, smooth. *Spore germination* of *Ustilago*-type (Ito, 1936: 17).

Hosts: *Bouteloua filiformis* (Fourn.) Griff., *Eragrostis* spp. (principal hosts), and *Sporobolus australasicus* Domin.

Known distribution: cosmopolitan. On *Sporobolus australasicus* Domin. only in Australia. There are variations in the density and coarseness of spore ornamentation between collections and on various host plants. An extreme of this is represented by *Macalpinomyces spinulosus*, which I am recognising as a separate species.

The generic place of *Macalpinomyces spermophorus* was obscure for a long time. It was described as *Ustilago*. Moesz (1921:63), based on the presence of sterile cells between the spores, a short, central columella in, and a peridium around the sori, transferred it into the genus *Sphacelotheca*. Now, it is known that *Sphacelotheca*, within the order Microbotryales, occurs only on members of the dicotyledonous Polygonaceae. The characters of our fungus fit very well with those of *Macalpinomyces*, hence its transfer into this genus.

5. *Macalpinomyces spinulosus* (L. Ling) Vánky, **comb. nov.**(Figs. 9, 13-14)
Basionym: ≡ *Ustilago spinulosa* L. Ling, Sydowia 7: 154 (1953). — Type on *Sporobolus patulus* Hack. (= *S. paniculatus* (Trin.) Th. Dur. and Schinz), Sierra Leone, summit of Picket Hill, 18 November 1951, T.S. Jones. (Holotype IMI 48887, isotypes BPI 166739!, HUV 17398!).

Sori (Fig. 9) in some ovaries of an inflorescence, broadly ellipsoidal, 1–1.5 mm in length, covered by a thin peridium of host and fungal origin which later ruptures disclosing the dark brown, powdery mass of spores. *Spores* (Figs. 13, 14) globose, ovoid, ellipsoidal, 7–10 x 7.5–11.5 µm, yellowish-brown, wall even, provided with sparsely situated, coarse, conical spines, 0.5–1 µm high; spore profile sparsely serrate; in SEM, between the spines, finely, sparsely verrucose. *Sterile cells* in groups, single cells subpolyhedrally irregular, rarely globose or ellipsoidal, 8–12 µm long, hyaline; wall thin, ca. 0.5 µm, smooth.

Host: *Sporobolus paniculatus* (Trin.) Th. Dur. and Schinz (*S. patulus* Hack.).

Known distribution: Africa (Sierra Leone).

6. *Macalpinomyces sporoboli* (Tracy and Earle) Vánky, **comb. nov.** (Figs. 10, 15-16)
Basionym: ≡ *Ustilago sporoboli* Tracy and Earle, Bulletin of the Torrey Botanical Club 23: 211 (1896), (not *U. sporoboli* Ellis and Everhart, 1897a: 282). **—** Type on *Sporobolus junceus* (Michaux) Kunth, USA, Mississippi, Columbus, 12 October 1895, M.S. Tracy and S.F. Earle. (Holotype BPI 166743!, isotypes BPI 166740!, 166741 and 194454; Topotype collected on 16 October1895, BPI 166742!, badly damaged by insects).

Sori (Fig. 10) in a few, hypertrophied ovaries of an inflorescence, ovoid, with a short, acute tip, laterally slightly compressed, ca. 1(–2) x 1.5–2.5(–3) mm, covered by a first green, later brown peridium, composed of an outer layer of the pericarp and an inner layer of sporogenous hyphae, enclosing the dark brown, first agglutinated, later semi-powdery mass of spores. *Spores* (Figs. 15, 16) globose to ellipsoidal, yellowish- to dark reddish-brown, 10.5–13(–13.5) x 11–14.5(–16) µm, including the densely situated, 1–2(–2.5) µm high, broadly conical, coarse spines; spore profile coarsely serrate. *Sterile cells* between the spores few, smaller than the spores, hyaline, smooth.

Host: *Sporobolus junceus* (Michaux) Kunth.

Known distribution: N. America (USA). Known only from the type locality.
The spores differentiate within the hyaline mass of sporogenous hyphae, in which the first elongated cell contents become shorter and thicker, finally spherical, each enclosed by a

thick, hyaline, amorphous mass of the gelatinised hyphal wall. During maturation, the hyaline spore initials increase in size, become ornamented and coloured whereas the surrounding hyaline mass decreases and finally disappears. The peculiar spore ornamentation of *M. sporoboli* is very similar to that of *M. elionuri-tripsacoidis* Vánky (on *Elionurus*), and of *M. ewartii* (McAlpine) Vánky and R.G. Shivas (on *Sorghum* spp.).

7. *Sporisorium hwangense* Vánky and C. Vánky, in Vánky, Mycotaxon 74: 194 (2000). (Figs. 17, 20-21)
Type on *Sporobolus panicoides* A. Rich., Zimbabwe, Matabeleland North Prov., Hwange (Wankie) National Park, Main Camp, Sedina Water Hole, alt. ca. 930 m., 6 March 1999, C. and K. Vánky. (Holotype HUV 18888!, isotypes BPI 746887 and in Vánky, Ust. exs. no. 1059).

Sori (Fig. 17) in all ovaries of an inflorescence, comprising also the inner floral organs, cylindrical, 0.5–1 x 3–8 mm, first covered by a whitish to pale yellowish-brown peridium composed of fungal cells which are arranged in tightly packed rows, and partially also of an external layer of host cells. The peridium ruptures longitudinally from its apex disclosing the dark brown, granular-powdery mass of spore balls intermixed with sterile cells surrounding a slender, simple, central columella of the length of the sorus. *Spore balls* (Figs. 20, 21) variable in shape and size, subglobose, ellipsoidal, pyriform, elongate or irregular, 30–70 x 30–100 µm, dark reddish-brown to opaque, rather permanent, composed of numerous spores. *Spores* (Figs. 20, 21) ellipsoidal to subpolyhedrally irregular, 7–11 x 8–12(–13) µm; outer spores dark reddish-brown with a ca. 1 µm thick wall, densely verruculose-echinulate on the free surface which appears finely serrulate in median view; inner spores lighter coloured, thin-walled (ca. 0.5 µm), apparently smooth. *Sterile cells* (Fig. 20) usually single, globose to ellipsoidal, 6–13 x 7–15 µm, hyaline; wall 1–3 µm thick, smooth.

Host: *Sporobolus panicoides* A. Rich.

Known distribution: Africa (Zimbabwe). Known only from the type locality.

8. *Sporisorium saharianum* (Trotter) Karatygin, in Karatygin and Azbukina, Opredelitel' gribov SSSR, etc.: 78 (1989). Figs. 18, 22-23)
≡ *Sorosporium saharianum* Trotter, in Saccardo and Trotter, Annales Mycologici 11: 413 (1913). **—** Type on *Aristida pungens* Schreber [= *Sporobolus pungens* (Schreber) Kunth], Libya, Tripoli, dunes near Sdun (Sliten), 25 April 1913, A. Trotter. (Holotype PAD!, isotype BPI 195123).

Sori (Fig. 18) comprise the whole inflorescence, branches of the inflorescence or single spikelets, several cm or only a few mm long, covered by a thin, yellowish-brown peridium enclosing the agglutinated or granular-powdery mass of spore balls surrounding one or several columellae. *Spore balls* (Figs. 22, 23) variable, mostly irregular, often elongated, 25–100 x 30–180 µm, dark reddish-brown to subopaque, composed of tens to hundreds of spores which separate by pressure. *Spores* (Figs. 22, 23) variable in shape and size, rounded subpolyhedrally irregular, elongated, more rarely globoid or ellipsoidal, 8–13.5 x 9–16(–17.5) µm, yellowish-brown; wall even to slightly uneven, 0.5–1 µm thick, densely, finely punctate-verruculose; spore profile smooth to very finely serrulate. *Sterile cells* not seen.

Host: *Sporobolus pungens* (Schreber) Kunth (*Aristida pungens* Schreber).

Known distribution: Africa (Libya).

9. *Tilletia sporoboli* Vánky, Mycotaxon 74: 194 (2000). (Figs. 19, 24-25)
Type on *Sporobolus festivus* A. Rich. (det. K.E. Bennett, SRGH), Zimbabwe, Midlands Prov., 15 km NW of Zvishavane, alt. ca. 1020 m., 1 March 1999, C. and K. Vánky. (Holotype HUV 18880!, isotypes BPI 746883, IMI 380468 and S).

Sori (Fig. 19) in some ovaries of the inflorescence, globoid to broadly ellipsoidal, ca. 1 mm in diameter, covered by the first green, later brown pericarp which ruptures irregularly disclosing the reddish-brown, granular mass of spores intermixed with sterile cells. *Spores* (Figs. 24, 25) subglobose, ovoid, ellipsoidal to slightly irregular, 13–18 x 16–21 µm, pale yellow to pale reddish-brown, provided with blunt, 1.5–2.5 µm high, coarse, somewhat irregular, conical or frustra-of-pyramid-like warts. *Sterile cells* (Figs. 24, 25) globose to ellipsoidal, variable in size, 10–22 x 12–24 µm, hyaline; wall 1.5–3 µm thick, smooth.

Host: *Sporobolus festivus* A. Rich.

Known distribution: Africa (Zimbabwe). Known only from the type locality.

10. *Tranzscheliella hypodytes* (Schltdl.) Vánky and McKenzie, Smut fungi of New Zealand: 156 (2002). (Figs. 26, 29-30)

≡ *Caeoma hypodytes* Schlechtendal, Flora Berolinensis, Pars 2. Cryptogamia: 129 (1824).

≡ *Ustilago hypodytes* (Schltdl.) Fries, Systema Mycologicum. Vol. 3, sect. 2: 518 (1832).

≡ *Erysibe hypodytes* (Schltdl.) Wallroth, Flora Cryptogamica Germaniae, Pars II, 4: 216 (1833).

≡ *Uredo hypodytes* (Schltdl.) Desmazières, Annales des Sciences Naturelles; Botanique, Sér. 2, 13: 182 (1840).

≡ *Cintractia hypodytes* (Schltdl.) Maire, Bulletin de la Societé Botanique de France 53: CXCVIII (1906). **—** Lectotype (design. by Hirschhorn, 1947: 74) on *Elymus arenarius* L. [= *Leymus arenarius* (L.) Hochst.], Germany, near Berlin, October 1884, P. Sydow; isolectotypes in Rbh., Fgi. eur. no. 3201, HUV 3784!

= *Ustilago sporoboli* Ellis and Everhart, Bulletin of the Torrey Botanical Club 24: 282 (1897a), (later homonym; not *U. sporoboli* Tracy and Earle, 1896: 211, *q.e. Macalpinomyces sporoboli*).

≡ *Ustilago funalis* Ellis and Everhart, Bulletin of the Torrey Botanical Club 24: 457 (1897b), (nom. nov. pro *U. sporoboli* Ellis and Everhart). **—** Type on *Sporobolus cryptandrus* Gray, USA, Colorado, foothills of the Rocky Mountains, July 1895, J.C. Cowen, (*n.v.*; syn. in Zundel, 1953: 168).

Further taxonomic synonyms are: = *Ustilago agrestis* Syd.; = *U. athenae* Maire; = *U. bromi-erecti* Cif.; = *U. hypodytes* var. *lolii* Thümen; = *U. lygei* Rabenh.; = *U. nummularia* Speg.; = *U. spegazzinii* Hirschh.; = *U. stipicola* Speg.; = *U. sumnevicziana* Lavrov (comp. Vánky, 1994: 361).

Sori (Fig. 26) in culms as a blackish-brown, semi-agglutinated to powdery spore mass surrounding the upper internodes (extending from the basal part of the internode sometimes to the next node) and occasionally in the axis of an abortive inflorescence. Sori at first protected by the leaf-sheath, finally more or less naked. Upper internodes and leaves of host usually stunted. Infection systemic, inflorescences usually abortive. *Spores* (Figs. 29, 30) globose, subglobose to ovoid, occasionally elongated, irregular or slightly compressed, 3.5–5.5 x 4–6(–7) µm, medium to dark olivaceous-brown; wall smooth, ca. 0.5 µm thick, usually with an inconspicuous, hyaline, smooth or finely punctate-verruculose cap at the poles; in SEM densely and minutely verruculose on the whole surface. *Spore germination* results in slender, septate (three- or four-celled, four-nucleate) basidia developing lateral, ramifying, septate, uninucleate branches. On nutrient media, these branches produce clumps of aerial "sporidia" (Bornhövd, 1936: 84, figs. 5–6; Dietz, 1956; Ingold, 1983: 583, fig. 9; 1987: 471, fig. 1).

Hosts: many grass species belonging to at least 35 genera, including *Sporobolus cryptandrus* (Torr.) A. Gray, and *S. agrostoides* Chiov. (*S. filipes* Napper).

Known distribution: cosmopolitan. On *Sporobolus* in North America (USA) and Africa (Kenya).

All material of *Tranzscheliella hypodytes* on "*Sporobolus*" I have seen was fragmentary, not allowing the identification of the host plants.

11. *Ustilago deformis* L. Ling, Sydowia 7: 152 (1953). (Figs. 27, 31-32)
Type on *Sporobolus patulus* Hack. (= *S. paniculatus* (Trin.) Th. Dur. and Schinz),, Sierra Leone, summit of Picket Hill, 18 November 1951, T.S. Jones. (Holotype IMI 48887, isotype HUV 17416!).

Sori (Fig. 27) forming pustules on the basal part of congested leaves on the top of sterile shoots, and on the distal part of the stem, 1–2 mm in diameter or larger by confluence, first covered by the epidermis which ruptures, disclosing the blackish-brown, powdery mass of spores. *Spores* (Figs. 31, 32) usually ovoid or ellipsoidal, slightly flattened, rarely globoid, 7–10.5 x 8–13 µm, dark yellowish-brown; wall uneven, thinner on the flattened sides, 0.5–1 µm thick, moderately densely echinulate; spore profile finely serrulate.

Hosts: *Sporobolus paniculatus* (Trin.) Th. Dur. and Schinz (*S. patulus* Hack.), *S. piliferus* (Trin.) Kunth.

Known distribution: Africa (Sierra Leone), Asia (Nepal).

12. *Ustilago peruviana* (Zundel) Vánky, **comb. nov.** (Figs. 28, 33-34)
Basionym: ≡ *Sphacelotheca peruviana* Zundel, Mycologia 34: 124 (1942). **—** Type on *Sporobolus virginicus* (L.) Kunth, Peru, Paracas Bay near Pisco, 1912, coll. H.O. Forbes, comm. A. Chase. (Holotype BPI 195082!, isotype BPI 194900!).

Sori (Fig. 28) destroying some ovaries of an inflorescence, globoid, slightly flattened, less than 1 mm in diameter, often with a short acute tip bearing remnants of the stigmata, more or less hidden by the floral envelopes and covered by a greenish-brown peridium composed of an outer layer of the pericarp and an inner layer of sporogenous hyphae, enclosing the agglutinated, later probably powdery, dark brown mass of spores. *Spores* (Figs. 33, 34) globose, subglobose, ovoid to long ellipsoidal, 4–5.5 x 5–7(–8) µm, yellowish-brown; wall even, ca. 0.5 µm thick, apparently smooth, in SEM sparsely low verruculose. *Sterile cells* absent.

Host: *Sporobolus virginicus* (L.) Kunth.

Known distribution: S. America (Peru). Known only from the type locality.

As in the case of *Ustilago utahensis* (see below), Zundel (1942: 124) considered this smut to be a *Sphacelotheca* species. We now know that species of *Sphacelotheca* are restricted to host plants in the Polygonaceae. In his description, Zundel wrote: "Sori . . . hard, . . . covered by a delicate whitish membrane that disintegrates into delicate sterile cells that soon collapse, spore mass agglutinated; sterile cells tinted olivaceous-yellow with a thick almost hyaline epispore, globose to subglobose, often irregular, chiefly 7–8 µ diameter, delicate and soon collapsing;". Judged from the study of the scanty, apparently immature type material, and from the original description, I conclude that what Zundel considered to be sterile cells are actually immature spores. Consequently, *Sphacelotheca peruviana* belongs to the genus *Ustilago*, rather than to *Sporisorium*; there are no spore balls or spore ball initials, columella/ae. Sterile cells are also lacking.

13. *Ustilago sporoboli-indici* L. Ling, Mycological Papers No. 11: 7 (1945). (Figs. 35A, B, 36, 39-40)
Type on *Sporobolus indicus* (L.) R. Br., China, Szechwan Prov., Chengtu, 12 September 1940, L. Ling. (Holotype IMI 501, isotypes BPI 166745, 196295, HUV 14063!; Topotype collected on 1 October1940, L. Ling, BPI 196293!).

= *Entyloma sporoboli* Castellani and Graniti, in Graniti, Nuovo Giornale Botanico Italiano, N.S., 57: 252 (1950). **—** Type on *Sporobolus indicus* R. Br. var. *laxus* Nees, Eritrea,

Seraé, Mai Felasi, 24 October 1938, F. Di Martino. (Holotype FL, isotype BPI 176675!; syn. by Ling, 1953: 154, confirmed).

Sori (Figs. 35A, B) in the leaves, leaf sheaths and stems of sterile shoots, forming short or long, bullate, lead-coloured striae, at first covered by the epidermis which early ruptures exposing the blackish-brown, semiagglutinated to powdery mass of spores which are scattered. The leaves become perforated or rupture longitudinally into fascicles. More rarely sori occur also on the floral axis or spikelets of weakly developed and deformed inflorescences. *Spores* (Fig. 39, 40) rather variable in shape and size, globose, subglobose, ovoid to long ellipsoidal, (5.5–)6.5–9.5 x (6.5–)7–11.5 µm, yellowish-brown; wall even, 0.5–0.8 µm thick, from apparently smooth to finely punctate or finely, moderately densely verrucose-echinulate which does not affect the spore profile. *Spore germination* (Fig. 36; on water-agar, at room temperature, in 1 day) results in 4-celled basidia measuring 1.5–2.5 x 35–45 µm. On the basidia, on short sterigmata, ovoid to long ellipsoidal basidiospores are produced measuring 1.5–2.5 x 5–14 µm. In 2 days, after conjugation of basidiospores or apparently without conjugation, long, ca. 1.5 µm wide, infection(?) hyphae developed.

Hosts: *Sporobolus africanus* (Poir.) Robyns and Tournay [*S. capensis* (Willd.) Kunth], *S. elongatus* R. Br., *S. indicus* (L.) R. Br., and its var. *laxus* Nees, *S. pyramidalis* P. Beauv.

Known distribution: Africa (Eritrea, Rep. of South Africa, Uganda, Zambia), Asia (China), Philippines.

14. *Ustilago sporoboli-tremuli* T.S. Ramakrishnan and K. Ramakrishnan, Proceedings of the Indian Academy of Science, Part B, 28: 58 (1948). (Figs. 37, 41-42)
Type on *Sporobolus tremulus* Kunth, India, Tamil Nadu, Coimbatore, Chettipalayam, 17 July 1934, N.K. Naidu. (Holotype HCIO 12113, isotypes BPI 166746, HUV 17347!).

Sori (Fig. 37) in the basal, swollen part of distal leaf sheaths of short, congested shoots, 1–3 mm long, covered by the epidermis. Spore mass blackish-brown, semiagglutinated to powdery. *Spores* (Figs. 41, 42) subglobose, ovoid, ellipsoidal or slightly irregular, 13–17 x 14.5–19(–20) µm, yellowish-brown; wall even, ca. 1 µm thick, finely, densely verrucose which just affects the spore profile.

Host: *Sporobolus tremulus* Kunth.

Known distribution: Asia (India). Known only from the type collection.

15. *Ustilago utahensis* (Zundel) Vánky, **comb. nov.** (Figs. 38, 43-44)

Basionym: ≡ *Sphacelotheca utahensis* Zundel, Mycologia 34: 125 (1942). **—** Type on *Sporobolus airoides* (Torr.) Torr. (= misnamed *S. patens* Swallen, teste K. Vánky), USA, Utah, Garfield Co., Escalante Mountains, 20 June 1932, coll. M. Stanton 770, comm. J.A. Stevenson. (Holotype BPI 195057!, isotype BPI 192090!).

Sori (Fig. 38) in all ovaries of an inflorescence, globoid to ovoid, with a short acute tip, ca. 1 x 1–2 mm, first covered by a peridium composed of an outer layer of the pericarp and an inner layer of irregular chains of septate, sporogenous hyphae in different stages of maturation. At maturity, the peridium ruptures irregularly, disclosing the dark brown, first agglutinated, later powdery mass of spores. No columella. *Spores* (Figs. 43, 44) globose, subglobose, ellipsoidal, 8–10.5 x 9–11(–12) µm, yellowish-brown; wall even, ca. 0.5 µm thick, sparsely, finely verrucose; spore profile almost smooth. *Sterile cells* absent.

Host: *Sporobolus patens* Swallen.

Known distribution: N. America (USA). Known only from the type locality.

Zundel (1942: 125) considered this smut to be a *Sphacelotheca* species because of the presence of supposed sterile cells, resulting from the peridium, "sterile cells", which are "globose to elongated, often irregular, hyaline, 7-14 µm long, smooth;" What Zundel

considered to be sterile cells are actually immature spores (see also *Ustilago peruviana* above).

The host plant of *Ustilago utahensis* is definitely not *Sporobolus airoides* (Torr.) Torr., as given originally. Judged from the two smutted inflorescences in BPI, it is most probably *S. patens* Swallen.

16. *Ustilago vilfae* G. Winter, Hedwigia 22: 2 (1883a); Bulletin of the Torrey Botanical Club 10: 7 (1883b). (Figs. 45, 48-49)
Type on *Vilfa vaginiflora* Torr. [= *Sporobolus vaginiflorus* (Torr.) Wood], USA, Pennsylvania, Chester Co., autumn 1881, coll. Martin Geo (Ellis no. 3729). (Holotype NY, isotype BPI 169423, devoid of sori).
= *Ustilago hilariae* Ellis and Tracy, Journal of Mycology 6: 77 (1890). **—** Type on *Hilaria jamesii* (Torr.) Benth., USA, New Mexico, Albuquerque, 17 June 1887, S.M. Tracy, BPI 160870, BPI 160871! (syn. by Fischer, 1953: 316).
= *Tilletia subfusca* Hume, Proceedings of the Iowa Academy of Science 9: 235 (1902). **—** Type on *Sporobolus neglectus* Nash, USA, Iowa, Spirit Lake, 15 November 1892, coll. J.C. Arthur. (Holotype BPI 173883; syn. in Zundel, 1953: 217).

Sori (Fig. 45) in the inflorescence, transforming it into a dark brown, semiagglutinated to powdery spore mass, 1–3 x 4–10 mm, more or less hidden by the distal leaf sheaths, but sori may also comprise the basal part of the uppermost, congested leaf sheaths and are then larger, or they may appear on the distal part of the stems as vesicles, covered by the epidermis. *Spores* (Figs. 48, 49) subglobose, ovoid or ellipsoidal, 12–14.5 x 13.5–16 µm, yellowish-brown; wall even, ca. 1 µm thick, evidently echinulate; spore profile serrulate.

Hosts: *Hilaria cenchroides* H. B. K., *H. jamesii* (Torr.) Benth., *H. mutica* (Buckl.) Benth., *Sporobolus neglectus* Nash, *S. vaginiflorus* (Torr.) Wood (*Vilfa vaginiflora* Torr.).

Known distribution: N. America (USA).
Zundel (1953: 165 and 217) treated *Ustilago vilfae* and *U. hilariae* as two separate species, whereas Fischer (1953: 316) considered them to be synonyms. Indeed, no differences in sorus or spore morphology could be seen between specimens on *Sporobolus* and *Hilaria*. *Ustilago vilfae* on *Lasiurus sindicus* Henrard [= *L. hirsutus* (Forssk.) Boiss.], reported by Agarwal *et al*. (1977: 206) from India, Western Rajastan (HCIO 32094!) represents *Sporisorium desertorum* (Thümen) Vánky.

Ustilago schlechteri is not on *Sporobolus*

There are several confusions regarding *Ustilago schlechteri* Henn. The type specimen, described on "*Sporobolus* sp." from South Africa, was destroyed by fire in Berlin, 1943. The original description is incomplete. This led Zundel (1953: 198) to give a more detailed description of this species, based on a specimen, identified by him as *U. schlechteri*, collected in South Africa, Natal Prov., by A.O.D. Mogg, on *Sporobolus indicus* (L.) R. Br. (PREM 11644, = BPI 166238! and 195260!). However, this collection represents another species, *U. sporoboli-indici* L. Ling. (det. K. Vánky). A topotype was found in BPI (166237), containing the tip of a single infected plant of "*Sporobolus* sp.", but with very typical sori. The host identity cannot be verified in lack of a healthy plant. Recently, during a survey of *Sporobolus* diseases in South Africa, a few hundred kms from the type locality of *U. schlechteri*, R.G. Shivas and the author collected a smut fungus on *Enneapogon scoparius* Stapf., with the same type of characteristic sori. A comparison of the spores confirmed that they represent the same fungus. This means that the host plant of *U. schlechteri* is not a *Sporobolus* sp. but *Enneapogon* cf. *scoparius*, which is common in that part of South Africa. The description below is based on the neotype, and on the specimens collected recently, edited in Vánky, Ust. exs. no. 1189.

Ustilago schlechteri Hennings, Hedwigia 34: 325 (1895). (Figs. 46-47, 50-53)

Type on "*Sporobolus* sp." (= misnamed *Enneapogon* cf. *scoparius* Stapf, teste K. Vánky), South Africa, Transvaal (= North-Western Prov.), Naboomfontein, 4300 ft., 23 May 1895, R. Schlechter (type destroyed in B). Neotype (designated here), collected in 1894 by Schlechter (= topotype; BPI 166237!).

Sori (Figs. 46, 47) in the basal part of the uppermost two (rarely one), congested leaf sheaths, swollen, ovoid, globoid or cylindrical with tapered distal part, 1.5–3.5 mm wide, together 3–7(–12) mm long, continued in filiform leaf blades. There often is an appendage with minute, scale-like floral remnants on the top of the distal sorus. Sori covered by the first green, later greyish-brown epidermis, which ruptures irregularly at maturity, disclosing the blackish-brown, powdery mass of spores. No columella, no sterile cells. Infection systemic, all shoots of an infected plant affected. Rarely, a few shoots escape infection, producing inflorescences. *Spores* (Figs. 50–53) globose, ovoid, ellipsoidal, elongated to slightly irregular, variable in size, 9.5–13 x 10–14.5(–15) µm, yellowish-brown; wall even, ca. 0.5 µm thick, sparsely, evidently, low echinulate; spore profile wavy to finely, sparsely serrulate.

Host: *Enneapogon scoparius* Stapf.

Known distribution: Africa (Rep. of South Africa).

Key to the smut fungi of *Sporobolus*

1. Sori surrounding upper internodes, naked ..... ***Tranzscheliella hypodytes***
1. Sori elsewhere, not naked..... 2

2. Sori in the leaves, or also in the stems..... 3
2. Sori in the ovaries, flowers or inflorescence..... 8

3. Sori as lead coloured pustules or streaks. Spore mass black, agglutinated 4
3. Sori not so. Spore mass more or less pulverulent..... 6

4. Spore wall 0.5–3(–3.5) µm thick, with very low tubercles. Exospore subhyaline to pale yellowish-brown ..... ***Jamesdicksonia major***
4. Spore wall 1.5–7(–8) µm thick, smooth. Exospore olivaceous-brown 5

5. Sori as swollen, elongated spots or striae. Spores mostly rounded-irregular, 7–19 µm long, pale olivaceous-brown..... ***Jamesdicksonia sporoboli***
5. Sori rounded or ellipsoidal. Spores mostly elongated-irregular, 11–20(–28) µm long, dark olivaceous-brown ..... ***Jamesdicksonia tremuli***

6(3). Sori as bullate striae. Spores 7–11.5 µm long..... ***Ustilago sporoboli-indici***
6. Sori on the basal part of uppermost leaves, swollen or bullate..... 7

7. Spores 8–13 µm long..... ***Ustilago deformis***
7. Spores 14.5–19(–20) µm long..... ***Ustilago sporoboli-tremuli***

8(2). Sori in the whole inflorescence 9
8. Sori in the flowers or ovaries..... 10

9. Peridium, columella, spore balls present. Spores densely, finely punctate-verruculose ..... ***Sporisorium saharianum***
9. Peridium, columella, spore balls absent. Spores evidently echinulate..... ***Ustilago vilfae***

10. Sori in the flowers. Columella and spore balls present..... ***Sporisorium hwangense***
10. Sori in the ovaries. Columella and spore balls absent..... 11

11. Spores 5–7(–8) µm long, in LM smooth ..... ***Ustilago peruviana***
11. Spores larger, ornamented 12

12. Sterile cells absent. Spores 9–11(–12) µm long, finely verruculose; spore profile almost smooth..... ***Ustilago utahensis***
12. Sterile cells usually present. Spores of various sizes, evidently to coarsely ornamented; spore profile serrulate or serrate 13

13. Spores 16–21 µm long..... ***Tilletia sporoboli***
13. Spores smaller..... 14

14. Spores 11–14.5(–16) µm long..... ***Macalpinomyces sporoboli***
14. Spores 7.5–11(–13) µm long..... 15

15. Spores moderately densely verrucose-echinulate. Spore profile finely serrulate..... ..... ***Macalpinomyces spermophorus***
15. Spores sparsely, coarsely echinulate. Spore profile sparsely serrate .... ***Macalpinomyces spinulosus***

HOST – PARASITE LIST

(*S.* = *Sporobolus*)

S. "*airoides*" = *S. patens – Ustilago utahensis*
S. africanus – Ustilago sporoboli-indici
S. agrostoides – Tranzscheliella hypodytes
S. arabicus = *S. marginatus – Jamesdicksonia major*, *J. sporoboli*
S. argutus = *S. pyramidatus – Jamesdicksonia major*
S. asperifolius = *Muhlenbergia asperifolia – Tilletia asperifolia*
S. auriculatus = *Muhlenbergia arenacea – Ustilago striiformis*
S. australasicus – Macalpinomyces spermophorus
S. brockmanii – Jamesdicksonia sporoboli
S. capensis = *S. africanus – Ustilago sporoboli-indici*
S. cordofanus – Jamesdicksonia major
S. cryptandrus – Tranzscheliella hypodytes
S. diander – Jamesdicksonia tremuli
S. elongatus – Ustilago sporoboli-indici
S. festivus – Tilletia sporoboli
S. filipes = *S. agrostoides – Tranzscheliella hypodytes*
S. gracillimus = *Muhlenbergia filiformis – Tilletia montana*
S. indicus – Jamesdicksonia sporoboli, *Ustilago sporoboli-indici*
S. indicus var. *laxus – Ustilago sporoboli-indici*
S. ioclados – Jamesdicksonia major
S. junceus – Macalpinomyces sporoboli
S. marginatus – Jamesdicksonia major, *J. sporoboli*
S. neglectus – Ustilago vilfae
S. panicoides – Sporisorium hwangense
S. paniculatus – Macalpinomyces spinulosus, *Ustilago deformis*
S. patens – Ustilago utahensis
S. patulus = *S. paniculatus*
S. piliferus – Ustilago deformis
S. pungens – Sporisorium saharianum
S. pyramidalis – Ustilago sporoboli-indici
S. pyramidatus – Jamesdicksonia major
S. spicatus – Jamesdicksonia major
S. tremulus – Jamesdicksonia tremuli, *Ustilago sporoboli-tremuli*
S. vaginiflorus – Ustilago vilfae
S. virginicus – Ustilago peruviana
S. wallichii – Jamesdicksonia tremuli
S. "sp." = *Enneapogon* cf. *scoparius – Ustilago schlechteri*

FUNGUS NAMES

(valid names in bold face)

agrestis Ustilago = ***Tranzscheliella hypodytes***
asperifolia Tilletia
athenae Ustilago = ***Tranzscheliella hypodytes***
bromi-erecti Ustilago = ***Tranzscheliella hypodytes***
cheoana Sphacelotheca = ***Macalpinomyces spermophorus***
deformis Ustilago
desertorum Sporisorium
Eballistra
elionuri-tripsacoidis Macalpinomyces
Entyloma
ewartii Macalpinomyces
funalis Ustilago = ***Tranzscheliella hypodytes***
hilariae Ustilago = ***Ustilago vilfae***

hwangense Sporisorium
hypodytes Caeoma = ***Tranzscheliella hypodytes***
hypodytes Cintractia = ***Tranzscheliella hypodytes***
hypodytes Erysibe = ***Tranzscheliella hypodytes***
hypodytes Tranzscheliella
hypodytes Uredo = ***Tranzscheliella hypodytes***
hypodytes Ustilago = ***Tranzscheliella hypodytes***
hypodytes Ustilago var. *lolii* = ***Tranzscheliella hypodytes***
Jamesdicksonia
kusanoana Ustilago = ***Macalpinomyces spermophorus***
lygei Ustilago = ***Tranzscheliella hypodytes***
major Jamesdicksonia
majus Entyloma = ***Jamesdicksonia major***
majus Melanotaenium = ***Jamesdicksonia major***
Melanotaenium
montana Tilletia
nummularia Ustilago = ***Tranzscheliella hypodytes***
orientalis Ustilago = ***Macalpinomyces spermophorus***
peruviana Sphacelotheca = ***Ustilago peruviana***
peruviana Ustilago
Phragmotaenium
saharianum Sorosporium = ***Sporisorium saharianum***
saharianum Sporisorium
schlechteri Ustilago
spegazzinii Ustilago = ***Tranzscheliella hypodytes***
spermophora Sphacelotheca = ***Macalpinomyces spermophorus***
spermophora Ustilago = ***Macalpinomyces spermophorus***
spermophorus Macalpinomyces
spinulosa Ustilago = ***Macalpinomyces spinulosus***
spinulosus Macalpinomyces
sporoboli Entyloma = ***Macalpinomyces sporoboli-indici***
sporoboli Jamesdicksonia
sporoboli Macalpinomyces
sporoboli Melanotaenium, (H.S. Jackson) Thirum., Whitehead and O'Brien = ***Jamesdicksonia sporoboli***
sporoboli Melanotaenium, (Thirum. and M.C. Sriniv. = ***Jamesdicksonia tremuli***
sporoboli Tilletia
sporoboli Tolyposporella = ***Jamesdicksonia sporoboli***
sporoboli Ustilago, Ellis and Everhart = ***Tranzscheliella hypodytes***
sporoboli Ustilago, Tracy and Earle = ***Macalpinomyces sporoboli***
sporoboli-indici Ustilago
sporoboli-tremuli Ustilago
stipicola Ustilago = ***Tranzscheliella hypodytes***
striiformis Ustilago striiformis
subfusca Tilletia = ***Ustilago vilfae***
sumnevicziana Ustilago = ***Tranzscheliella hypodytes***
tremuli Jamesdicksonia
utahensis Sphacelotheca = ***Ustilago utahensis***
utahensis Ustilago
vilfae Ustilago

Acknowledgements

For generous assistance during a shared 3-week survey of diseased *Sporobolus* in South Africa, in December 2002, my thanks go to Dr. Roger G. Shivas (BRIP, Queensland Dept. of Primary Industries, Australia), and to Arne Witt (South African Field Station, Queensland Dept. of Natural Resources and Mines, Pretoria). I thank Dr. Eric H.C. McKenzie (Auckland, New Zealand) for reading the manuscript and correcting my English.

I would also like to acknowledge financial support for the *Sporobolus* survey in South Africa from Meat and Livestock Australia Limited, and kind support from Dr. Bill Palmer (Alan Fletcher Research Station, Queensland Dept. of Natural Resources and Mines, Australia). I am grateful to the directors and curators of BPI, HCIO, IMI, PAD and PC for loan and also exchange of herbarium specimens.

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Figure legends

Fig. 1A. Sori of *Jamesdicksonia major* on a leaf of *Sporobolus spicatus* (from isotype), **B.** Sori of *Jamesdicksonia tremuli* on a leaf of *Sporobolus wallichii* (from HCIO 20563), **C.** *Jamesdicksonia sporoboli* on a leaf of *Sporobolus junceus* (from isotype). Bar = 1 cm.

Fig. 8. Sori of *Macalpinomyces spermophorus* in swollen ovaries of *Sporobolus australasicus* (Vánky, Ust. exs. no. 1111). Habit and enlarged a healthy spikelet (below) and three spikelets with sori of different maturity. Bars = 1 cm, and 1 mm for enlargement.

Fig. 9. A sorus of *Macalpinomyces spinulosus* in a swollen seed of *Sporobolus paniculatus* (from type). To the left a healthy spikelet with a seed. Bar = 1 mm

Fig. 10. Sori of *Macalpinomyces sporoboli* in some swollen ovaries of an inflorescence of *Sporobolus junceus* (from holotype). Enlarged is a healthy spikelet, a healthy seed and three mature sori. Bars = 1 cm, and 1 mm for enlargement.

Fig. 17. Sori of *Sporisorium hwangense* in all flowers of *Sporobolus panicoides* (from holotype). To the left a healthy inflorescence with seeds. Bar = 1 cm.

Fig. 18. Sori of *Sporisorium saharianum* on *Sporobolus pungens* (from holotype). To the left sori in branches of an inflorescence, to the right in single spikelets. Bar = 1 cm.

Fig. 19. Sori of *Tilletia sporoboli* in some, considerably swollen ovaries of *Sporobolus festivus* (from holotype). Enlarged a spikelet with a sorus. Bars = 1 cm, and 1 mm for enlargement.

Fig. 26. Sori of *Tranzscheliella hypodytes* on *Nassella mucronata* (Kunth) L. Pohl (Ecuador; Vánky, Ust. exs. no. 937).

Fig. 27. Sori of *Ustilago deformis* on the stem and basal part of leaves of *Sporobolus paniculatus* (from isotype). Bar = 3 mm.

Fig. 28. Sori of *Ustilago peruviana* in two seeds of *Sporobolus virginicus* (from type). Bar = 1 mm.

Fig. 35A-B. Sori of *Ustilago sporoboli-indici* on the leaves of **A.** *Sporobolus pyramidalis* (Uganda; HUV 20019), and on **B.** *Sporobolus africanus* (South Africa; Vánky, Ust. exs. no. 1192). Each with a healthy inflorescence. Bars = 1cm.

Fig. 36. Sori of *Ustilago sporoboli-tremuli* in the basal, swollen part of distal leaf sheaths of *Sporobolus tremulus* (from type). Bar = 2 mm.

Fig. 37. Sori of *Ustilago utahensis* in all ovaries of an inflorescence of *Sporobolus patens* (from type). Habit, and enlarged a spikelet with a sorus. Bars = 1 cm, and 2 mm for enlargement.

Fig. 45. Sori of *Ustilago vilfae* in two inflorescences of *Sporobolus neglectus* (Ellis and Everhart, Fgi. Colomb. no. 2197). Bar = 1 cm.

Fig. 46. Sori of *Ustilago schlechteri* in the basal part of uppermost leaf sheaths of "*Sporobolus* sp." (from topotype). Bars = 1cm, and 2 mm for enlargement.

Fig. 47. Sori of *Ustilago schlechteri* in the basal part of uppermost leaf sheaths and often also in remnants of the inflorescence of *Enneapogon scoparius* Stapf. (from Vánky, Ust.

exs. no. 1189). Three three infected shooths and, to the left, a healthy inflorescence. Bar = 1cm.

Figs. 2, 3. Spores of *Jamesdicksonia major* on *Sporobolus pyramidatus*, in LM and SEM (from Ciferri, Mycofl. doming. exs. no. 96, as *Tolyposporella sporoboli*).
Figs. 4, 5. Spores of *Jamesdicksonia sporoboli* on *Sporobolus indicus*, in LM and SEM (from holotype).
Figs. 6, 7. Spores of *Jamesdicksonia tremuli* on *Sporobolus wallichii*, in LM and SEM (from HCIO 20563). Bars = 10 µm.

Figs. 11, 12. Spores and sterile cells of *Macalpinomyces spermophorus* on *Sporobolus australasicus*, in LM and SEM (from Vánky, Ust. exs. no. 1111).
Figs. 13, 14. Spores of *Macalpinomyces spinulosus* on *Sporobolus paniculatus*, in LM and SEM (from holotype).
Figs. 15, 16. Spores of *Macalpinomyces sporoboli* on *Sporobolus junceus*, in LM and SEM (from holotype). Bars = 10 µm.

Figs. 20, 21. Spore balls, spores and a sterile cell (arrow) of *Sporisorium hwangense* on *Sporobolus panicoides*, in LM and SEM (from holotype).
Figs. 22, 23. Spores and spore balls of *Sporisorium saharianum* on *Sporobolus pungens*, in LM and SEM (from holotype).
Figs. 24, 25. Spores and sterile cells of *Tilletia sporoboli* on *Sporobolus festivus*, in LM and SEM (from holotype). Bars = 10 µm.

Figs. 29, 30. Spores of *Tranzscheliella hypodytes* on *Elymus repens* (L.) Gould, in LM and SEM (New Zealand, Vánky, Ust. exs. no. 785).
Figs. 31, 32. Spores of *Ustilago deformis* on *Sporobolus paniculatus*, in LM and SEM. (from isotype).
Figs. 33, 34. Spores of *Ustilago peruviana* on *Sporobolus virginicus*, in LM and SEM. (from holotype). Bars = 10 µm.

Figs. 39, 40. Spores of *Ustilago sporoboli-indici* on *Sporobolus pyramidalis*, in LM and SEM (from isotype).
Figs. 41, 42. Spores of *Ustilago sporoboli-tremuli* on *Sporobolus tremulus*, in LM and SEM (from isotype).
Figs. 43, 44. Spores of *Ustilago utahensis* on *Sporobolus patens*, in LM and SEM (from holotype). Bars = 10 µm.

Figs. 48, 49. Spores of *Ustilago vilfae* on *Sporobolus neglectus*, in LM and SEM (from Ellis and Everhart, Fgi. Colomb. no. 2197, HUV 4947).
Figs. 50, 51. Spores of *Ustilago schlechteri* on "*Sporobolus* sp.", in LM and SEM (from topotype).
Figs. 52, 53. Spores of *Ustilago schlechteri* on *Enneapogon scoparius* Stapf, in LM and SEM (from Vánky, Ust. exs. no. 1189). Bars = 10 µm.

Appendix D

Cultivation and Infection Experiments with *Sporobolus africanus* and *Sporobolus pyramidalis* and their Pathogen, the Smut Fungus *Ustilago sporoboli-indici*

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Introduction

During the survey for pathogens of Sporobolus in South Africa (4-29th December 2002), together with Dr. Roger Shivas and Arne Witt, numerous collections of the smut fungus *Ustilago sporoboli-indici* were made, mainly on *Sporobolus pyramidalis* but also a few collections on *Sporobolus africanus*. Some healthy inflorescences with seeds were also collected in South Africa, although in December most plants were still only flowering. Both grass species occur as weeds in eastern Australia. Because this pathogen showed a good potential as biocontrol agent, its biology and way of infection should be studied.

Description of the Experiments and the Results

1. Germination of the Sporobolus seeds

Seeds of dried and pressed herbarium specimens of *Sporobolus africanus* and *Sporobolus pyramidalis*, collected in December 2002 in South Africa, were used for different germination experiments. Due to the time of collecting, only a few ripe seeds of *S. pyramidalis* (the mainly infected species in S.A.) were available and therefore seeds of *Sporobolus africanus* were used in most experiments. Many of the collections had to be frozen at –25°C for three days, because of heavy attack by insects, feeding on the smut spores. Frozen specimens are marked by * in the following text.

1.a *Sporobolus africanus* on filter paper

Seeds of *Sporobolus africanus* (Plettenberg Bay, 16.12.02, not *), without counting and without selecting seeds from the surrounding glumes, were put on wet filter paper in a covered Petri dish, kept at room temperature (22°C day, 18°C night). Daylight was prolonged by a special plant light in the morning and evening to make a 12 hours day. After four days, the first three seeds germinated. On the fifth day, further 16, on the seventh day 18 seeds germinated. Until the tenth day only three more seeds germinated, none after this date.

Respectively one day after they germinated, the 40 seedlings were planted in flowerpots with commercial garden-mould, that had before been sterilised by heating it in the microwave. The flower pots were covered with a plastic lid during the night to get higher humidity. Three seedlings died a few days later, probably because their roots were injured when planting them.
From the beginning of the experiment (18.1.) until 10.5.2003 the remaining 37 plants grew well. They are now 25 cm tall and are put outside without extra light or heating.

1.b *Sporobolus pyramidalis* on water agar

Selected seeds of *Sporobolus pyramidalis* (Nylstroom, 8.12.2002, not *) were washed for 30 minutes in boiled water with 0.01% Amisept and rinsed three times with boiled water, to

sterilise the surface of the seeds. The seeds were then put on water-agar (see below) in Petri dishes (instead of wet filter paper). The idea was, to infect the seedlings on the agar and be able to observe the germinating spores on or next to the plants directly in the stereoscope. Unfortunately, no seeds germinated.

1.c *Sporobolus pyramidalis* on filter paper

200 ripe seeds of *Sporobolus pyramidalis* (Nylstroom, 8.12.2002 not *) were selected, separated from the glumes and put in a Petri dish on wet filter paper. After three weeks, still no seed germinated.

1.d *Sporobolus africanus* directly on soil

360 selected, ripe seeds of *Sporobolus africanus* (Plettenberg Bay, 16.12, not *) were put directly on soil in flower pots. After 10 days, 34 seeds have germinated (= 9%), none after that time.

2. Germination of the spores of *Ustilago sporoboli-indici*

Spores of several collections of *Ustilago sporoboli-indici* (collected in December 2002 in South Africa) were germinated in Petri dishes with water agar, enriched with very little malt and, to prevent growth of bacteria, the antibiotic Chloramphenicol was added. This culture medium was supplied by the Mycological Laboratory of Dr. Frans Spaaij, Tübingen.

2.a Spores of *Ustilago sporoboli-indici* from *Sporobolus pyramidalis*

Spores not frozen:
Spores of *Ustilago sporoboli-indici* from *Sporobolus pyramidalis* (Bukawe Nature Reserve, 8.12.2002, not *) were mixed with a little boiled water and poured onto three Petri dishes with water agar (see above). After 20 hours at 22°C practically all spores have germinated. In Petri dishes no. 1 and 2, four celled basidia measuring 1.5-2.5 x 35-45 µm, were observed. On the basidia, on short sterigmata, numerous ovoid to long ellipsoidal basidiospores were produced, measuring 1.5-2.5x 5-14 µm. In two days, after conjugation of basidiospores (or apparently without conjugation), long, c. 1.5 µm wide, infection(?) hyphae developed. (For drawings of the germination see "The smut fungi (Ustilaginomycetes) of Sporobolus (Poaceae)" Kálmán Vánky, Fungal Diversity, in press)
For unknown reasons, in Petri dish no. 3, basidia and thick hyphae were observed, no basidiospores at all.

Spores frozen:
Spores of *Ustilago sporoboli-indici* from *Sporobolus pyramidalis* (Nelspruit, 20.12.2002, *) and *Sporobolus pyramidalis* (Bukawe Nature Reserve, 8.12.2002, *) were dusted on water agar. After 18 hours c. 90% of the spores have germinated with four celled basidia with numerous basidiospores. Very few infection hyphae could also be seen. Their number increased the next day.

2.b Spores of *Ustilago sporoboli-indici* from *Sporobolus africanus*

Spores of *Ustilago sporoboli-indici* from *Sporobolus africanus* (Plettenberg Bay, 16.12.2002, *) were dusted on water agar. After 18 hours c. 90% of the spores have germinated with four celled basidia, producing numerous basidiospores. A few infection hyphae were also seen.

3. Infection experiments

Seeds, germinating seeds and seedlings of both Sporobolus species were "infected" at different ages and in different ways with spores or germinated spores of *Ustilago sporoboli-indici* from different collections (* and not *) of both hosts.

("Infected" in quotation marks is used for the different procedures of bringing the seeds or plants together with smut spores or germinated spores, regardless if the plants showed symptoms later and can therefore really be called infected.)

3.a "Infecting" *Sporobolus africanus* plants with spores from *Sporobolus pyramidalis*

The 40 *Sporobolus africanus* plants from experiment 1.a were divided into four groups and "infected", when they were c. one week old:
group 1: the 10 plants, that germinated first, were not "infected" with spores directly after planting them, but left as healthy control.
group 2: the next 10 plants were dusted with dry spores, taken from *Sporobolus pyramidalis* (Bukawe Nature Reserve, 8.12.2002, not *).
group 3: upon 10 plants (c. 5-6 days old) a suspension of basidiospores in water was dropped. This suspension was produced by rinsing the Petri dish no. 2 (from experiment 2.a) with a few ml of boiled water and carefully scratching the spores of the agar with the back of a scalpel. The suspension was mixed well in a pipette before dropping it directly onto the seedlings.
group 4: upon 10 plants (c. 5-6 days old) a suspension of hyphae (from Petri dish no. 3) in water was dropped (procedure see group 3).

The first infected plant was observed six weeks after germination and five weeks after "infecting" the plant. It was a plant of group 3, dropped with a suspension of basidiospores. When showing the first symptoms, the plant was 12 cm tall and the fourth of five leaves showed a 15 mm long sorus along the midrib and four 1-10 mm long sori on the leaf sheath. The sori appear as dark striae, containing the smut spores, covered by the epidermis. About four weeks later, the epidermis ruptures and the blackish-brown, semi-agglutinated to powdery mass of spores is exposed. All later following leaves were also visibly infected. Even one of the four secondary shoots developed a 1.5 mm long sorus.

The chronologically sixth of all observed infections appeared in the same group, but 16 weeks after germination. The main shoot seemed healthy, also four of the five secondary shoots. But the fourth leaf and leaf sheath of one of the secondary shoots developed four small sori, 1-8 mm long.

3.b "Infecting" *Sporobolus africanus* plants with spores from *Sporobolus africanus*

I. A second lot of the *Sporobolus africanus* seeds (Plettenberg Bay, 16.12.02, not *) was germinated in a Petri dish with wet filter paper. Within one week, 42 seeds germinated. As soon as they germinated, the seedlings were planted into soil and "infected" before they were three days old. For "infecting", the spores of the same collection were used, that the seeds for this experiment were taken from.

-one third was left "uninfected" as control

-one third was dusted with dry spores

-onto one third a suspension of germinated spores with basidiospores was dropped. Of this last group, four plants died within the next days.

II. The same infection scheme was used for the 34 seedlings of experiment 1.d (*Sporobolus africanus* (Plettenberg Bay, 16.12.02, not *), directly on soil), but for "infection", a frozen portion of the same collection was used. Also in the third group of this experiment, five seedlings did not survive the first three days after "infection".

The third visibly infected plant belonged to the group of *Sporobolus africanus*, grown directly on soil. The symptoms showed four weeks after the plant was dropped with a suspension of basidiospores, when it was two days old. The plant was stunted, only half as tall as the others, the fourth leaf was deformed and heavily attacked. A few days later, this plant died.

The fourth infected plant also belonged to this group. It showed the symptoms five weeks after "infection". It was not stunted. The leaf sheath of the fourth leaf and all following leaves

were attacked. The plant so far did not develop any secondary shoots, as most of the healthy plants did.

3.c Locally "infecting" (inoculating) *Sporobolus africanus* with germinating spores from *Sporobolus africanus*

When the plants of experiment 3.a, group 1 (not "infected" control) were four weeks old, 7 of the 10 were scratched with a needle near the base and in this wound, germinating spores from *Sporobolus africanus* (Plettenberg Bay, 16.12.2002, not *) were smeared to produce local infection.

The second infection became obvious eight weeks after germination and four weeks after inoculating the plant. The leaf sheath of the sixth and the leaf blade of the seventh leaf showed six sori. The leaf was deformed and crinkled.. The following leaves of the main shoot did not show symptoms of infection, but two of the three secondary shoots did.

3.d Germination of seeds and spores together

I. Seeds of *Sporobolus pyramidalis* (Nelspruit, 20.12.2002, *) and spores of the same collection were mixed in water and poured onto filter paper in a Petri dish.

After five days, only four seeds have germinated. They were planted into soil one or two days after germination.

II. Seeds of *Sporobolus pyramidalis* (Bukawe, 8.12.2002, not*) and spores of the same collection were mixed dry and spread on wet filter paper in a Petri dish.

27 seeds germinated within five days. They were planted into soil one or two days after germination.

III. Seeds of *Sporobolus africanus* (Plettenberg Bay, 16.12.2002, not*) and spores of the same collection (but *) were mixed in water and poured onto filter paper in a Petri dish. Within five days 24 seeds have germinated. They were planted into soil one or two days after germination.

IV. Seeds of *Sporobolus africanus* (Plettenberg Bay, 16.12.2002, not*) and germinated spores (24 hours old) of the same collection were mixed in water and poured on filter paper in a Petri dish. The germination rate was much lower than in experiment 3.c III, where the same seeds were used. Of the 13 planted seedlings only 9 survived. Nearly all of them were very weak and pale, they also grew slower than all the others.

The fifth infected plant was a result of the experiment 3.d III. It was heavily infected and stunted (half the size of the healthy ones). All leaves were attacked. The sori were 1-4 cm long, but c. one month after they appeared, they were still covered by the epidermis. The plant practically stopped growing after showing the symptoms.

Discussion

General remarks:

Altogether 169 plants were grown (surviving until 10.5.2003), 138 *Sporobolus africanus* plants and 31 *Sporobolus pyramidalis* plants. 32 plants of *Sporobolus africanus* were left "uninfected" as control, none of *Sporobolus pyramidalis*, due to the low number of available plants.

Of the 137 "infected" plants, so far only six (= 4.5%) showed symptoms in form of sori on the leaves and leaf sheaths. All infected plants belonged to the species *Sporobolus africanus*,

none to *Sporobolus pyramidalis*, which is (as all results of these experiments) statistically not relevant, because we had 106 S.a./31 S.p. "infected" plants.

The experiment is still continued and for the number of visibly infected plants, it must always read "so far". As a surprise, e.g. the sixth infected plant appeared 10 weeks after the first within the same experiment.

The investigated Sporobolus species are perennial plants and it is impossible to predict, how long it can take for the infection to show. In the literature (e.g. Fischer and Holton, Biology and control of the smut fungi), usually the cultivated, annual grasses like wheat, barley, oat or corn, were studied. For these plants the results of "infection" become visible within a few weeks.

A further practical problem was, that the seeds and seedlings of Sporobolus are much more difficult to handle than e.g. wheat or corn, simply because they are much smaller.

To experiment 1: Germination of seeds:

As already mentioned, the germination rate of the Sporobolus seeds was very low, especially for *Sporobolus pyramidalis*. The reasons could be wrong time of collecting the seeds (not ripe enough?), wrong time and conditions for germinating them (European winter, indoors, artificial light and heating, wrong soil, no resting period ???.....). Some seeds were even frozen, although (against all expectations) this did not seem to make much difference.

All *Sporobolus pyramidalis* plants grew much faster and taller than the *Sporobolus africanus* plants. The now ten weeks old plants measure 42-45 cm (compared to 25 cm of the 15 weeks old *Sporobolus africanus* plants).

After the seedlings survived the first week, there were no losses of older plants.

To experiment 2: Germination of spores:

Quite contrary to the seeds, all samples of spores from both host plant species germinated extremely well with 90-100%! It did not even matter, that they had to be frozen, which was expected to have a bad effect on a (sub)tropical fungus. The germination rate was a little better, when a suspension of spores in water was poured on water agar, than when the dry spores were dusted directly on the agar. The reason probably just is, that the spores are not distributed as evenly and flatly on the agar in the second version, but some spores in bigger lumps did not get into direct contact with the medium.

To experiment 3: Infecting Sporobolus with *Ustilago sporoboli-indici*:

The most interesting and obvious result of the Sporobolus "infection" experiments certainly is, that four of the six visibly infected plants occurred after dropping a suspension of germinated spores onto the 1-6 days old seedlings. This seems to be the most effective way of infecting the plants. It obviously does not matter, if the spores for "infecting" are taken from the same host species, or, as in experiment 3.a, are taken from another host species.

When the suspension was dropped on very young seedlings (experiment 3b I+II, group 3), nearly one third died within the next days, because they did not seem to be strong enough to cope with the infection. It is even more surprising, that the surviving seedlings did not show a higher rate of visible infection (so far?). None of the plants died, when they were dropped with the suspension, when they were c. one week old (experiment 3.a). The older plants were stronger and already better established in the soil after planting. But from both experiments (3.a and 3.b II) resulted two visibly infected plants. This indicates, that the fear was unjustified, that the plants may only be susceptible to infection by the smut in a very early

stage. In future experiments, better results might be obtained by "infecting" the plants only after 5-7 days, to avoid loosing to many seedlings.

The same phenomenon was observed in experiment 3.c IV, where the seeds were mixed with germinating spores. The germination rate was low, the seedlings were weak, pale and slow growing, many of them died early. But from the surviving ones, not a single one showed symptoms!

The two other infections do not allow a conclusion, except that probably it is also possible to get an infection by locally inoculating older plants and by mixing seeds and spores in water.

It can not be absolutely excluded, that the seeds came into contact with spores before or while collecting. The healthy plants were collected in the same areas as the infected ones.

If the experiments are continued, it would be best to take seeds from Australian specimens, that have certainly never been in contact with the smut. If the seeds are harvested at the optimal time, the germination rate should also be better. Cultivation during European summer would also be easier than starting to germinate (sub)tropical grasses in January.

Literature

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