Towards the Management of *Ustilago kamerunensis* H Sydow and Sydow, a Smut Pathogen of Napier Grass (*Pennisetum purpureum* Schum.) in Kenya.

GRAHAM FARRELL

A thesis submitted in partial fulfilment of the requirements of the University of Greenwich for the Degree of Doctor of Philosophy

This research programme was carried out in collaboration with the Kenya Agricultural Research Institute

November 1998

I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

G Farrell

Dr S A Simons
(supervisor)

ABSTRACT

Infection by headsmut, caused by *Ustilago kamerunensis,* is the major cause of biomass reduction in Napier grass (*Pennisetum purpureum*). In Kenya, this grass is the primary source of fodder for zero grazed livestock, which are an important component of small-holder farm incomes in high potential areas of the country.

Studies were conducted on the biology and ecology of the pathogen, with the objective of developing technologies for management of the disease. A survey in Kiambu District, central Kenya, of Napier grass plots was used to construct a model of smut incidence. Ten agro-ecological and environmental parameters were factored into the model but only altitude, using a quadratic function, was significant. Napier grass at altitudes between 1800 and 2000masl is particularly at risk from the disease. This was the first smut disease correlative model to make use of environmental factors. The presence of the fungus was demonstrated inside the host, and descriptions of ustilospore and sorus characteristics were made. Ustilospores in soil remained viable for less than eight weeks, and those air dried for less than 14 weeks. The infection court was identified as being restricted to germinating buds, which were the only sites on which ustilospores gave rise to appressoria. On other host surfaces, and on agars, sporidia and hyphae were formed.

A disease severity scale was developed, related to biomass reduction in fresh weight of Napier grass stools, that can be used to assess losses in field situations. Using a spore dipping technique, resistance testing of locally available cultivars identified susceptible and resistant types. Evidence was also gained for the possibility of disease remission.

These investigations lead to the production of management options for farmers, that are discussed in view of the increasing importance of the disease in Kenya.

ACKNOWLEDGEMENTS

I express my sincere thanks to Sarah Simons of CAB International for her consistent supply of advice, encouragement and coffee during the course of this work. Bernie Briscoe and John Bridge, also of CABI, assisted with nematode authorities. Rick Coe of ICRAF provided guidance through the complexities of modelling and statistical analysis. Jackson Kung'u from KARI and Jim Waller of CABI introduced me to smut disease of Napier grass and helped with sourcing references and techniques. The success of the field survey was greatly enhanced by KARI driver Peter Njonjo, who helped with Kikuyu translation and field assistance.

I am particularly grateful to my wife, Tish Farrell, for her anthropological insights into the lives of Kenyan farmers. Without her continuous support the investigations reported here would not have been possible.

This project was completed under the second phase of the KARI/DFID National Agricultural Research Project, as part of the UK bilateral aid programme to Kenya, and this assistance is gratefully acknowledged. The views expressed herein are my sole responsibility and do not necessarily reflect the opinions of DFID or KARI.

Plant pathology is basically a practical exercise in problem solving...

W C James, 1983

TABLE OF CONTENTS

Declaration ii
Abstract iii
Acknowledgements iv
Table of contents v
Abbreviations viii

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 HOST, PATHOGEN AND FARMING SYSTEM

2.1 The host - the genus *Pennisetum* 5
2.1.1 *Pennisetum purpureum*, description and use as fodder 5
2.1.2 Other uses of *Pennisetum purpureum* 9
2.1.3 *Pennisetum purpureum* Schum. clones and hybrids 9
2.1.4 Pests and diseases of *Pennisetum purpureum* 14
2.1.5 *Pennisetum purpureum* as a weed 19

2.2 The pathogen cohort - smut fungi 19
2.2.1 Morphology of smut fungi and disease symptoms 21
2.2.2 Taxonomy of smut fungi 24
2.2.3 Host range of smut fungi 28
2.2.4 Smut diseases in Kenya 31
2.2.5 Generalised life cycle of smut fungi 31
2.2.6 The genus *Ustilago* 36
2.2.7 Hosts of *Ustilago* species 37

2.3 *Ustilago kamerunensis* 38

2.4 The Napier grass farming system - zero grazing 41

CHAPTER 3 NAPIER GRASS PEST AND DISEASE SURVEY

3.1 Introduction 43

3.2 Methods
3.2.1 Postal questionnaire 43
3.2.2 Field survey 46

3.3 Field survey results
3.3.1 Analysis of survey data 51
3.3.2 Modelling the probability of smut disease incidence . 60

3.4 Discussion 64

CHAPTER 4 THE PATHOSYSTEM

4.1 External morphology of smut-infected Napier grass 70

4.2 Internal morphology of smut-infected Napier grass
4.2.1 Methods 75
4.2.3 Results 75

4.3 Ustilospores of *Ustilago kamerunensis* 76

4.4 The sorus of *Ustilago kamerunensis* 77

4.5 Cultural characteristics of *Ustilago kamerunensis*
4.5.1 Ustilospore germination 80

4.6 Discussion 85

CHAPTER 5 EPIDEMIOLOGY OF *USTILAGO KAMERUNENSIS*

5.1 The infection court
5.1.1 Introduction 91
5.1.2 Methods 92
5.1.3 Results 93
5.1.4 Discussion 95

5.2 Spore survival
5.2.1 Introduction 97
5.2.2 Methods 100
5.2.3 Results 103
5.2.4 Discussion 108

5.3 Disease gradients and spore deposition
5.3.1 Introduction 114
5.3.2 Methods 115
5.3.3 Results 121
5.3.4 Discussion 122

CHAPTER 6 BIOMASS REDUCTION CAUSED BY *USTILAGO KAMERUNENSIS*

6.1 Introduction 125

6.2 Methods 129

6.3 Results 131

6.4 Discussion 140

CHAPTER 7 RESISTANCE OF NAPIER GRASS TYPES TO *USTILAGO KAMERUNENSIS*

7.1 Introduction 146

7.2 Methods 149

7.3 Results 151

7.4 Discussion 153

CHAPTER 8 DISEASE REMISSION

8.1 Introduction 157

8.2 Methods 157

8.3 Results 158

8.4 Discussion 160

CHAPTER 9 CONCLUSIONS 163

References 177

Appendices

ABBREVIATIONS

CBS	Central Bureau of Statistics
AEZ	Agro-ecological zone
df	degrees of freedom
DFID	UK Department for International Development (formerly ODA)
FAO	Food and Agriculture Organisation of the United Nations
FURP	Fertilizer Use Research Project
GPS	Global Positioning System
ILRI	International Livestock Research Institute
KARI	Kenya Agricultural Research Institute
LH	Lower Highland
masl	meters above sea level
MoLD	Ministry of Livestock Development
MoALDM	Ministry of Agriculture, Livestock, Development and Marketing
sd	standard deviation
se	standard error
sem	standard error of the mean
UH	Upper Highland
UM	Upper Midland
Unesco	United Nations educational, scientific and cultural organisation

CHAPTER 1 INTRODUCTION

Over the past 50 years many new technologies have been developed to increase production of tropical fodder crops. Work has concentrated mainly on production improvements through soil amendments, plant breeding, rangeland management and stock levels. Fodder pathogens and pests, however, have received scant attention from researchers, in comparison with the extensive work on crops grown for human consumption. Pathogens were regarded as relatively unimportant, and so pathology information on fodder crops was usually restricted to lists of pests and diseases. This situation began to change after a major outbreak of anthracnose disease on the legume *Stylosanthes*, which occurred in the 1970s in northern Australia and Florida. At the same time, tropical pasture programmes became more active in the centres of origin of pasture plants, particularly in Central and South America, and disease constraints to legume and grass production began to be recognized. These events stimulated the development of tropical pasture pathology as a discipline, although most efforts were directed at diseases in America and Australia (Lenné and Trutmann, 1994).

Similarly, in East Africa, work on fodder crops concentrated on soil fertility, varietal productivity and grazing management (Boonman, 1993). Crops such as maize and sorghum were primarily cultivated for human food; the availability of cereal stover for animal feeding was a secondary consideration. Plants

grown specifically for fodder rarely succumbed to serious pest or disease outbreaks, and fodders were grown in low- or no-input farming systems. For these reasons, plant pathological investigations of fodder and pasture species were rare and " *...their diseases have largely been ignored.*" (Mohamed-Saleem and Berhe, 1994).

The introduction of milk quotas in Europe has eliminated superfluous milk stocks, that had been exported cheaply to East Africa as powdered formulations, undercutting local markets. This, combined with recent market liberalisation in Kenya, means that milk production has become economically viable in the country. Milk from two dairy cows can yield an income of about US $180 per month. Kiambu District, north of Nairobi, is one of the high potential areas of Kenya, and 77 percent of households there keep dairy cattle. In a survey of dairy farms supplying the Nairobi milk market, over 40 percent of these households reported dairying as their major source of income (Staal *et al.*, 1997). In the country as a whole, 80 percent of milk is produced by small-holder farmers (Chavangi, 1987).

The most important fodder crop for Kiambu farmers is Napier grass (*Pennisetum purpureum* Schum.). Before the 1960s, livestock were free range and fed on the grass on roadsides and river banks. However, increasing competition for land since then has led to the introduction of cut-and-carry feeding of penned livestock. This has resulted in farmland being given over to

Napier grass production, with a concomitant increase in the area under cultivation. In their survey of Kiambu District, Staal *et al.* (1997) found that 14 percent of households had taken up Napier grass cultivation over the previous decade, with the grass now occupying approximately 15 percent of all the arable land in Kiambu. Napier grass is regarded as just another farm crop, albeit a very low input one.

Although several plant pathogens have been described on Napier grass in Kenya (Lenné, 1990; Kung'u and Boa, 1997), they were seldom severe and rarely merited the attention of plant pathologists. The only control option offered by government advisors was to plough in the crop and replant. Nevertheless, given the large increase in Napier grass acreage, and the fact that the types available come from a very narrow genetic base, it was perhaps inevitable that a more persistent and serious disease would arise.

In the early 1990s such a disease was reported (Kung'u and Waller, in press). It was caused by a smut fungus, *Ustilago kamerunensis* H Sydow and Sydow, which is presumed to have spread from Tanzania or Uganda. The disease now infects the major regions of Napier grass production in Kenya. In contrast to existing foliar pathogens, this systemic pathogen considerably reduces plant biomass, and thus has a direct effect on the usefulness of Napier grass as livestock feed.

The investigation described here was thus instigated in response to a serious disease that threatens a major source of fodder for Kenyan dairy cattle. Dairy products make an important contribution to small-holder farm incomes and maternal and child health. Chavangi (1987) found that women are particularly important in Kenyan dairy production, contributing as they do 70-80 percent of the routine dairy work, including all activities connected with fodder.

The objectives of the work outlined in this thesis were to elucidate the basic biology and ecology of the smut pathogen and examine its relationships with the host. The ultimate aim was to contribute to the development of options for the management of Napier grass smut disease for Kenyan farmers.

CHAPTER 2 HOST, PATHOGEN AND FARMING SYSTEM

2.1 The host - the genus *Pennisetum* Rich. in Persoon

The genus *Pennisetum* is within the Gramineae (the grasses), in the sub-family Panicoidae, tribe Paniceae (Prain, 1934). The tribes are distinguished according to the position of the spikelet relative to the rachis, the regression of the basal floret and the predominance of the inflorescence (Häfliger and Scholz, 1980). The genera are differentiated on the basis of flower architecture and growth habit. *Pennisetum* comprises over 140 species, distributed in tropical and warm regions, and includes annual and perennial examples (Prain, 1934; Brunken, 1977). Napier grass is in *Pennisetum* section *Penicillaria*, which also contains the millets (*P. typhoides* Stapf & Hubbard and *P. americanum* Schumacher) that are important food crops in drier regions.

2.1.1 *Pennisetum purpureum*, description and use as fodder

Elephant grass is the common name of *Pennisetum purpureum* when referring to the grass occurring naturally. In its cultivated state it is also known as Napier grass, especially in Kenya (Boonman, 1993). It is indigenous to the Zambezi valley. The first record of the value of the grass dates from 1905, when Mynhardt sent specimens to the Zurich Botanical Gardens from northern Rhodesia (Boonman,1993). Melle (1918) described the grasses' habit and cultivation. It was named, however, after Colonel Napier of Bulawayo, who alerted the Agricultural Department to its usefulness (Boonman, 1993). Prain

(1934) refers to *P. purpureum* as *Napier's fodder*. Another species, *Pennisetum polystachion* (L.) Schult., is known as thin Napier grass (Lenné, 1990).

Prain (1934) described *P. pennisetum* as a robust perennial, often forming large bamboo-like clumps, sometimes spreading by long stolons with many nodes. The culms are erect, 2-8m high and 3cm in diameter at the base, and branched upwards. The stems are more or less hairy towards the inflorescence, up to 20-noded, the nodes being glabrous with a ring of long, stiff appressed hairs. The leaf blades are linear, tapering to a fine point, 0.5-1.5m long and 20-40mm wide, flat, dull green or sometimes glaucus or tinged with purple (hence the specific name *purpureum*), usually more or less hairy above and with a stout midrib. The inflorescence is cylindrical, 8-20cm (sometimes 30cm) long and 1.5-3cm in diameter. Spikelets are solitary or in groups of 2-4, especially in the lower part of the spike (Häfliger and Scholz, 1980). The seeds are freely produced and drop early (Purseglove, 1972), and so plants tend to be propagated vegetatively because of the difficulty of collecting the seeds. The chromosome number is 28. Anatomy of the species is also described by Brunken (1977), Polhill (1982) and Ibrahim and Kabuye (1988). Figure 1 shows the habit and major morphological features of *P. purpureum.*

Pennisetum purpureum is the dominant grass in the fertile crescent along the north of Lake Victoria and the western Rift Valley in Uganda, where there is a

mean annual rainfall of 1000-1500mm. It has also been introduced into South America, southern USA, the Middle East, India, South-East Asia and China, Australia and the Pacific Islands.

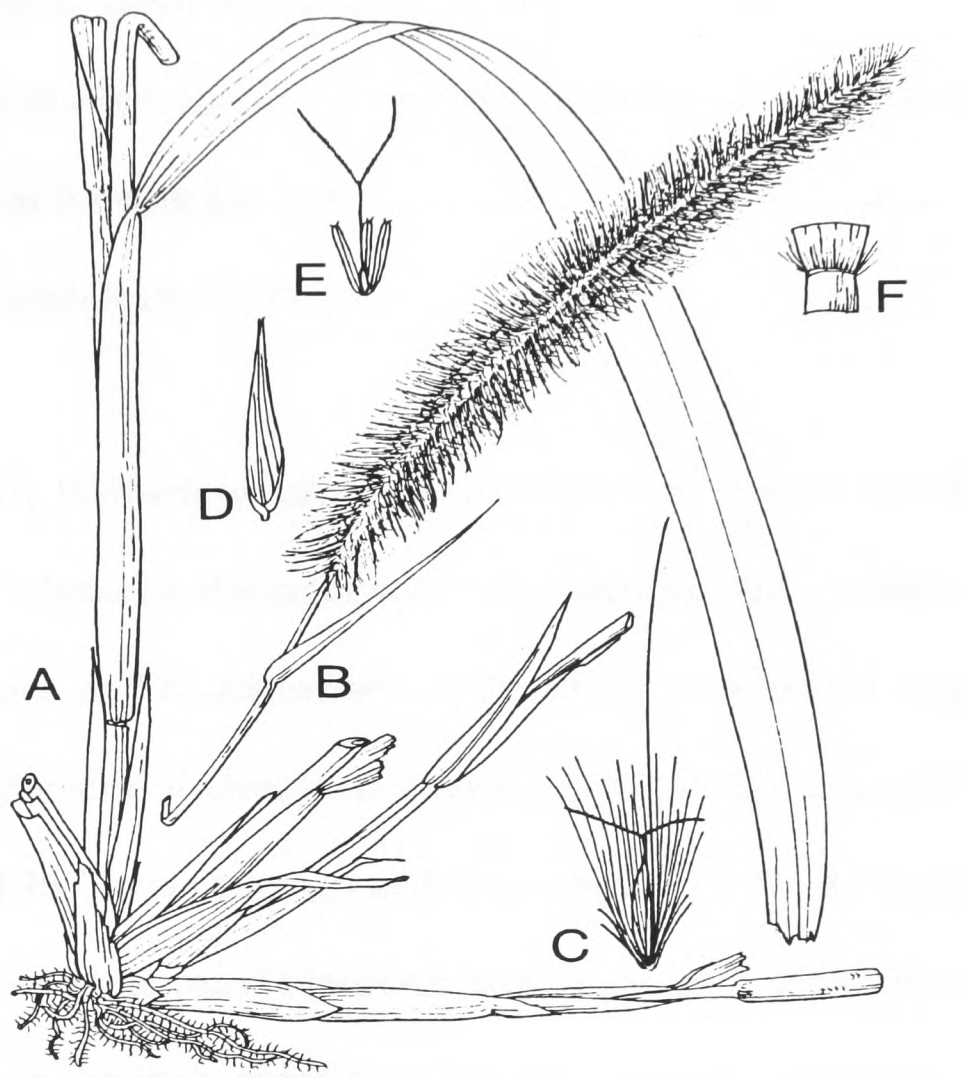


Figure 1. *Pennisetum purpureum*. A - habit; B - inflorescence; C - spikelet with bristles; D - spikelet without bristles; E - flower; F - ligule (from Skerman and Riveros, 1990).

The optimum growth temperature for Napier grass is 25-40°C, although it can grow at 15°C. It is very susceptible to frost. Its latitudinal limits are between 10°N and 20°S and it performs well from sea level up to 2000m. Napier grass

grows best in high rainfall areas (in excess of 1500mm per year), but its deep root system allows it to survive during dry periods. It does not tolerate flooding (Skerman and Riveros,1990).

The main use for Napier grass is animal fodder. The first cut is made about three months after planting of stem cuttings or root splits, and thereafter at intervals of six to eight weeks. It has the advantage of withstanding repeated cutting. Four to six cuts per year can produce 50-150 tonnes of green matter per hectare (Purseglove, 1972).

Bogdan (1977), Williams *et al.* (1980), Whiteman (1980) and Skerman and Riveros (1990) describe the cultivation and management of Napier grass in the tropics. The use and management of the grass as a fodder crop in Kenya is given by the Ministry of Livestock Development (MoLD, undated), Henderson and Preston (1957) and Onyango and Kevelenge (1987). If regularly fertilized, *P. purpureum* exhibits rapid regrowth and produces a high biomass which is very palatable in the leafy stage (van der Wouw *et al.*, in press), although it is best replanted every five or six years. Woodward *et al.* (1991) reported that it can also be made into silage for feeding during the dry season. The grass grows well with leguminous trees (Horne and Blair, 1991), or climbing legumes such as *Clitoria ternatea* L, thus increasing total yield (Mureithi *et al.*, 1995).

2.1.2 Other uses of *Pennisetum purpureum*

The first report of an alternative use for Napier grass was by Nye (1937), who noted that the grass was employed for soil regeneration on cotton in 1932 in Buganda (Uganda), after the failure of green manuring. Another early note, reporting experiments done in 1926, referred to the value of Napier grass as a mulch on coffee, again in Uganda (Tothill, 1940). Napier grass mulches have also been assessed in coffee in Kenya (McDonald, 1937; Anon., 1952). The wide range of applications of the grass is shown in Table 1. A comprehensive description of all uses of Napier grass is given by Boonman (1993).

2.1.3 *Pennisetum purpureum* clones and hybrids

Although reference is normally made to varieties, Napier grass types are truly clones (Boonman, 1993). In Kenya, the common forms of Napier grass are French Cameroon, Cameroon, Uganda hairless, Clone 13, Minna (a dwarf type), Gold Coast and Bana (Boonman,1993). Hybrids such as cumbu-Napier (*P. americanum* (=*P. glaucum* Stapf & Hubbard) x *P. purpureum*), and king grass (also known as bajra grass), *P. purpureum* x *P. typhoides*, have been produced to improve the fodder quality of the parents.

Stapf (1934) discussed the difficulties of distinguishing *Pennisetum* species, particularly in clarifying the relationships between cultivated and wild types. He gave the origin of the Penicillarias as Africa and their derivation from two sources - the tall, widely spread mesophytic *P. purpureum* and the xerophytic

group of smaller species, which ranges from The Gambia to the Red Sea.

Table 1. Uses of *Pennisetum purpureum*.

country	use	crop	reference
Australia	firebreaks, boiler fuel	sugarcane	Alexander, 1985
Brazil	mulch	lettuce	Nakagawa *et al.*, 1992
Brazil	mulch	maize, cowpea	Schoningh, 1985
Cuba	green manure	kenaf, *Hibiscus cannabinus* L	Sistachs *et al.*, 1990
Cuba	growth support	*Teramnus labialis* (L)	Matias & Matias, 1995
India	limit wilt disease	coconut	Thomas, 1988
Indonesia	activated carbon	oil palm production	Sudrajat & Hartadi, 1992
Indonesia	cover crop	rubber	Siagian & Sumarmadji, 1989
Kenya	intercrop	fuelwood	Jama *et al.*, 1991
Malawi	constituent of fish food	_____	Chikafumbwa *et al.*, 1993
Mauritius	production of *Stomoyxs nigra* (Diptera:Muscidae)	_____	Anon., 1974a
Nigeria	mulch	plantain	Salau *et al.*, 1992
Paraguay	ant bait when mixed with *aldrin*	_____	Robinson, 1979
Philippines	export as animal fodder to Japan	_____	Palacpac, 1985
not given	intercrop	plantain	Swennen & Wilson, 1985
	diagnosis of ratoon stunting disease	_____	Steindl, 1974; Perez *et al.*, 1981
not given	pathotyping *Erwinia chrysanthemi* Burkholder, McFadden and Dimock	_____	Rivera *et al.*, 1980
not given	paper pulp production	_____	Boonman, 1993
not given	erosion control	tea	Williams *et al.*, 1980

Stapf concluded that the wide and massed distribution of *P. purpureum* must have contributed to the appearance of hybrids between it, and cultivated forms. Attempts to classify *Pennisetum* solely on morphological characters have resulted in the generation of a large number of synonyms. Brunken (1977) lists

14 for *P. purpureum* alone in a systematic study of the subsection *Pennisetum*. Javier (1969) demonstrated significant differences in morphological traits in six accessions of Napier grass in the Philippines, which were sufficient to distinguish between them. Tcaceno and Lance (1992), in Brazil, identified 89 morphological characters in nine accessions. They concluded that it was possible to discriminate between accessions but admitted the presence of large variations between plants in the same accession. In Egypt, Hassan *et al.* (1983) measured vegetative characteristics in 23 accessions, but were only able to relate plant height, leaf width and stem diameter to accession. More recently, van der Wouw *et al.* (in press) compared 53 African Napier grass accessions using 28 morphological and agronomic characters. Using principal components analysis, six main groups could be identified based on morphological characters. However, only five groups could be identified using the agronomic factors. Combining morphological and agronomic characters gave six groups, showing the dominance of morphological characters in the clustering. Boonman (1993) stressed that qualitative differences were needed to distinguish varieties, and that hairiness of the leaf blade was the easiest parameter to assess. However, it is clear that considerable experience is needed to identify clones using morphological characteristics. Table 2 shows the characters used by Boonman (1993) to differentiate Kenyan clones of Napier grass.

The high levels of ambiguity in the definition of morphological characters using

Table 2. Botanical characters of some Napier grass clones in Kenya (after Boonman,1993).

clone	no. of stems at maturity (m^2)	stem diameter	hairs on leaf sheath	back "comb" of hairs on upper fringe of leaf sheath	long hairs on leaf blade near ligule	hairiness on upper surface of leaf blade	aerial tillering from nodes	flowering at Kitale[1]
Gold Coast[2] Bana[2] Ghana[2]	10-15	thick	2-3mm dense	very common	common	hairy to the touch; very dense short hairs	uncommon	very uncommon
French Cameroon[3]	15-20	thin	2-3mm not dense	uncommon	uncommon	not hairy to the touch; scattered short hairs	common	common
Clone 13[4]	30-40	very thin	3-4mm not dense	uncommon	very common	not hairy to the touch; scattered short hairs	very common	very common
Uganda hairless[5]	20-25	thin	2-3mm not dense	uncommon	uncommon; short hairs	not hairy to the touch; hairless to scattered short hairs	uncommon	uncommon

[1] Kitale agricultural research station is the main site of pasture research in Kenya.
[2] Palish colour; spreading habit; old leaves remain attached to stems; top leaves give stem top a fan-shaped appearance; leaf sheaths diverging which "flattens" the stem.
[3] Very fast establishment from cane cuttings. [4] Erect habit; very poor establishment from cane cuttings. [5] Erect habit; old leaves purplish; open clumps.

principal components analysis led Lowe *et al.* (in preparation), working in Kenya, to investigate additional marker systems. Of 19 accessions of Napier grass and its hybrids, using random amplified polymorphic DNA markers, they demonstrated a high degree of genetic variation between individuals. The cultivar Mott was clearly distinguished, whereas French Cameroon and Clone 13 were genetically very similar, not surprisingly since Clone 13 was originally derived from French Cameroon. Bana was slightly differentiated from Uganda hairless and Gold Coast, suggesting that Bana was not an interspecific hybrid between *P. typhoides* and *P. purpureum* but was actually a form of *P. purpureum.* This is substantiated by chromosomal studies reported by Karanja (1981), in which Bana types had 28 chromosomes, and not 21 as would be expected if they were hybrids. Figure 2 shows a proposed lineage of Napier grass varieties in Africa.

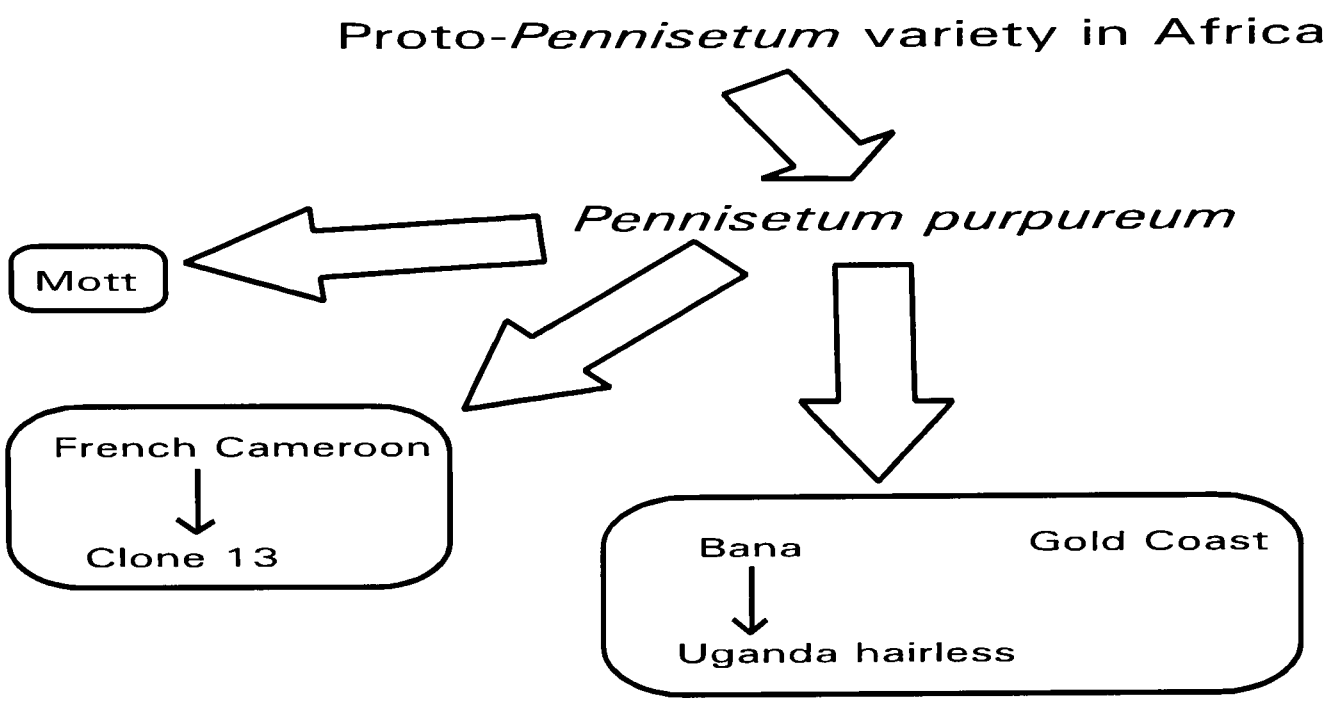


Figure 2. Possible derivation of and relationships between types of *Pennisetum purpureum* (types within rectangles are genetically similar (derived from Lowe *et al.*, in preparation)).

2.1.4 Pests and diseases of *Pennisetum purpureum*

Skerman and Riveros (1990) regarded the grass as being relatively free of serious pests and diseases. However, Lenné (1990) listed 66 fungal species in 38 genera that have been found on *P. purpureum*. Table 3 shows arthropods recorded on or associated with *P. purpureum*, whereas Table 4 lists fungi infecting the grass in Africa and Table 5 lists fungal records from other parts of the world. Bacteria, viruses and diseases of unknown aetiology are given in Table 6. None of these pests or diseases were considered of major importance by Boonman (1993). Apart from *U. kamerunensis*, the only other smut fungus recorded on Napier grass is *Tolyposporium penicillariae* Bref. in Zimbabwe (Lenné, 1990).

Table 3. Records of arthropods associated with *Pennisetum purpureum*.

organism	country	reference
insects and mites		
Abrostola triopsis (Lep.:Noctuidae)	Uganda	Le Pelley, 1959
Acigona ignefusalis (Lep.:Pyralidae)	Senegal	Gahukar, 1990
Agonscelis pubescens (Hem.:Pentatomidae)	Uganda, Tanzania	Le Pelley, 1959
Amrasca sp. (Hom.:Cicadellidae)	India	Sachan, 1980
Apogonalia grossa (Hom.:Cicadellidae)	Brazil	Menezes & De-Menezes, 1978
Bagrada cruciferarum (Hem.:Pentatomidae)	India	Singh Sandhu, 1975
Barbaropus cuprea (Col.:Languridae)	Uganda	Le Pelley, 1959
Borbo fatuellus (Lep.:Hesperidae)	Uganda	Le Pelley, 1959
Busseola fusca (Lep.:Noctuidae)	Ethiopia	Gebre-Amlak & Amlak, 1988
	Uganda	Ingram, 1958
B. phaia and *B. segeta*	Uganda	Le Pelley, 1959
Cataloipus oberthuri (Orth.:Acrididae)	Uganda	Le Pelley, 1959
Chilo zonellus (Lep.:Pyralidae)	Uganda	Ingram, 1958
Cicadulina sp. (Hom.:Cicadellidae)	India	Sachan, 1980
Coccus tenuivalvatus (Hem.:Coccidae)	Uganda	Le Pelley, 1959
Cropera testacea (Lep.:Lymantriidae)	Uganda	Le Pelley, 1959
Crorema fuscinotata (Lep.:Lymantriidae)	Uganda	Le Pelley, 1959
Dasychira pennatula (Lep.:Lymantriidae)	Uganda	Le Pelley, 1959
Diapalpus congregarius (Lep.:Lasiocampidae)	Uganda	Le Pelley, 1959
Diopsis apicalis (Dip.:Diopsidae)	Nigeria	Deeming, 1982
Diplognatha gagetes (Col.:Cetoniidae)	Uganda	Le Pelley, 1959
Duronia tricolor (Orth.:Acrididae)	Uganda	Le Pelley, 1959
**Epilachna similis* (Col.:Coccinellidae)	Ghana	Scheibelreiter & Inyang, 1974
Faureia milanjica (Orth.:Acrididae)	Uganda	Le Pelley, 1959
Helopeltis bergrothi (Hem.:Miridae)	Uganda	Le Pelley, 1959
Hybosorus orientalis (Col.:Scarabaeidae)	India	Patil & Veeresh, 1984
Hydrellia sp. (Dip.:Ephydridae)	Sierra Leone	Alghali & Domingo, 1982
Hypsotropa sp. nr. subcostella (Lep.:Pyralidae)	Uganda	Ingram, 1958
Lachnosterna (=*Holotrichia*) *consanguinea* (Col.:Scarabaeidae)	India	Bindra & Singh, 1971
Locusta migratoria migratoriodes (Orth.:Acrididae)	Uganda	Le Pelley, 1959
Lepidiota reuleauxi (Col.:Scarabaeidae)	Papua New Guinea	Kuniata & Young, 1992
Leucania insulicola (Lep.:Noctuidae)	Uganda	Le Pelley, 1959
Marasmia venilialis (Lep.:Pyralidae)	Uganda	Le Pelley, 1959
Melanitis leda (Lep.:Nymphalidae)	Uganda	Le Pelley, 1959
Metarctia flaviciliata (Lep.:Syntomidae)	Uganda	Le Pelley, 1959
Mocis latipes (Lep.:Noctuidae)	Brazil	Silva & Neves, 1984
Myllocerus undecimpustulatus	India	Sachan, 1980
var. *maculosus* (Col.:Curculionidae)	India	Sachan, 1980
M. cardoni	India	Sachan, 1980
Mythimna separata (Lep.:Noctuidae)	India	Singh & Chaudhary, 1988
M. loreyi	India	Singh & Chaudhary, 1987
Neomaskellia bergii (Hem.:Aleyrodidae)	Uganda	Le Pelley, 1959
	Réunion	Russell & Etienne, 1985
Nilaparvata lugens (Hem.:Delphacidae)	India	Zaheruddeen & Rao, 1988
Peregrinus maidis (Hem.:Delphacidae)	India	Chatterjee & Nimbalkar, 1977
Phragmatoecia pallens (Lep.:Cossidae)	Uganda	Le Pelley, 1959
Pterandrus sp. (Dip.:Trypidae)	Kenya	G N Kibata, *pers. comm.*
Rhopalosiphum maidis (Hem.:Aphidae)	India	Wadhi *et al.*, 1973
Rigema ornata (Lep.:Notodontidae)	Uganda	Le Pelley, 1959
Sesamia botanephaga (Lep.:Noctuidae)	Uganda	Ingram, 1958
S. calamistis	Uganda, Tanzania	Le Pelley, 1959
Sesamia sp. n., nr. *cretica*	Uganda	Ingram, 1958
S. griscens	Papua New Guinea	Young & Kuniata, 1992
S. oriaula	Uganda	Ingram, 1958
S. poebora	Uganda	Le Pelley, 1959
S. poephaga	Uganda	Ingram, 1958
S. nonagrioides	Cyprus	Krambias *et al.*, 1973
S. nonagrioides sub sp. *botanephaga*	West Africa	Bowden, 1976
Stenotus sp. (Hem.:Miridae)	Uganda	Le Pelley, 1959
Taphronata calliparea (Orth.:Acrididae)	Uganda	Le Pelley, 1959
Taragama butiti (Lep.:Lasiocampidae)	Uganda	Le Pelley, 1959
Tetranychus Dufour sp. (Aca.:Tetranychidae)	Kenya	Bogdan, 1977
Zonocerus variegatus (Orth.:Acrididae)	Uganda	Le Pelley, 1959
millipedes		
Haplothysanus oubanguiensis (Spirostreptida:Odontopygidae)	Central African Republic	Pierrard, 1969

**Henosepilachna* (=*Epilachna*) *hirta* Thunberg

Table 4. Records of fungi associated with *Pennisetum purpureum* in Africa (see also Table 12).

name	country	reference
Acremonium album Preuss	Tanzania	Lenné, 1990
Alternaria tenuissima (Nees ex Pers.) Wilts.	Kenya	Ondieki, 1973
Apiospora camptospora Penz. & Sacc.	Kenya	Nattrass, 1961
**Beniowskia sphaeroidea*	Zimbabwe	Mtisi & Milliano, 1993
(Kalchlbr. & Cooke) Mason	Kenya	Maher, 1936; Nattrass, 1941
	Malawi, Tanzania Uganda, Zimbabwe	Lenné, 1990
Cercospora Fresen. sp.	Malawi, Zambia Zimbabwe	Lenné, 1990
C. fusimaculans Atk.	Kenya	Nattrass, 1961
	Zambia	Lenné, 1990
C. oryzae Miyake	Nigeria	Adeoti & Adeniji, 1982
C. penniseti Chupp	Malawi	Lenné, 1990
C. sorghi Ell. & Ev.	Kenya	Nattrass, 1961
Didymosphaeria panici Hansf.	Kenya	Nattrass, 1961
	Tanzania, Uganda	Lenné, 1990
Drechslera S. Ito sp.	Uganda	Lenné & Trutmann, 1994
	Malawi	Lenné, 1990
D. (=*Helminthosporium*) *sacchari* (Butler) Subram. and Jain	Kenya	Bogdan, 1977
Fusarium Link sp.	Uganda	Lenné, 1990
Gloeocercospora sorghi Deighton	Tanzania	Lenné, 1990
Helminthosporium Link sp.	Ethiopia	Mengistu *et al.*, 1993
H. ocellum Faris (= *H. sacchari*)	tropics	Bogdan, 1977
Khuskia oryzae Hudson	Kenya, Tanzania	Lenné, 1990
Leptosphaeria bicolor D. Hawksw.	Kenya	Kaiser *et al.*, 1979
L. penniseticola Deighton	Sierra Leone	Lenné, 1990
Magnaporthe grisea (Herbert) M. E. Barr	Kenya	Lenné, 1990
Mycosphaerella Johanson sp.	Kenya	Ondieki, 1973
Myrothecium gramineum Li.	Togo	Lenné, 1990
Nigrospora state of *Khuskia oryzae* Hudson	Kenya	Gatumbi, 1985
N. sphaerica (Sacc.) Mason	Tanzania	Lenné, 1990
Periconia sacchari Johnston	Togo	Lenné, 1990
Phaeocystostroma sacchari var. *penniseti* B. Sutton	Zambia	Lenné, 1990
Phoma Sacc. sp.	Ethiopia	Mengistu *et al.*, 1993
Phyllachora minutissima (Welw. & Currey) A. L. Sim	Malawi	Lenné, 1990
P. pennisetina Zimm.	Sudan	Tarr, 1955
	Ghana, Guinea, Malawi, Nigeria, Sierra Leone Tanzania, Uganda	Lenné, 1990
Phyllosticta Pers. sp.	Malawi	Lenné, 1990
P. healdii Sprague	Kenya	Gatumbi, 1985
P. penicillariae Speg.	Uganda	Lenné, 1990
Puccinia penniseti Zimm.	Malawi	Lenné, 1990
P. substriata var. *penicillariae* Ramachar & Cumm.	Ghana, Malawi, Tz.	Lenné, 1990
Pyricularia grisea (=*Magnaporthe*) (Cooke) Sacc.	Kenya	Nattrass, 1961
Ramulispora alloteropis Thirum. & Narasimh.	Malawi, Nigeria Sudan, Tanzania	Lenné, 1990
Septoria Sacc. sp.	Malaysia, Tanzania	Lenné, 1990
S. penniseti Gonz. Frag. & Cif.	Uganda	Lenné, 1990
Tolyposporium penicillariae	Zimbabwe	Lenné, 1990
Trichonectria Kirschst. sp.	Togo	Lenné, 1990

* *Beniowskia sphaeroidea* (Kalchlbr. & Cooke) Mason (syn. *Clathrotrichum* Pat.1921). Synonyms of the common name are white rust, white mould, false mildew and snow mould.

Table 5. Records of fungi associated with *Pennisetum purpureum* outside Africa.

name	country	reference
Balansia claviceps Speg.	Puerto Rico, WI	Lenné, 1990
Beniowskia sphaeroidea	Mauritius	Lenné, 1990
Bipolaris sacchari (E. Butler) Shoem.	Jamaica, USA	Lenné, 1990
Cercospora fusimaculans Atk.	Australia	Lenné, 1990
Cochliobolus cynodontis Nelson	Malaysia, Trinidad	Lenné, 1990
C. eragrostidis (Tsuda & Ueyama) Sivan.	Malaysia	Lenné, 1990
C. herterostrophus (Drechsl.) Drechsl.	French Guyana	Lenné, 1990
C. intermedius Nelson	Malaysia	Lenné, 1990
C. lunatus Nelson & Haasis	Malaysia	Lenné, 1990
C. pallescens (Tsuda & Ueyama) Sivan.	Malaysia	Lenné, 1990
Colletotrichum graminicola (Ces.) Wilson	Brazil	Lenné, 1990
Coniothyrium fuckelii Sacc.	Peru	Lenné, 1990
Curvularia Boedijn sp.	Colombia, India	Lenné, 1990
C. leonensis M. B. Ellis	Malaysia	Lenné, 1990
Drechslera graminea (Schlect.) Shoem.	USA	Lenné, 1990
D. hawaiiensis M. B. Ellis	French Guyana	Lenné, 1990
D. poae (Baudys) Shoem.	Columbia	Lenné, 1990
D. (=*Helminthosporium*) *sacchari*	Puerto Rico	Burton, 1989
	USA	Sprague, 1950
	Jamaica	Lenné, 1990
Ephelis japonica Henn.	Puerto Rico	Lenné, 1990
Fusarium equiseti (Corda) Sacc.	Malaysia	Lenné, 1990
Fusicladium Bonorden sp.	Nicaragua	Lenné, 1990
Helminthosporium sp.	Antilles	Pauvert & Jacqua, 1974
	Guadalupe	Lenné, 1990
	St Kitts & Nevis	
	St Vincent, Trinidad & Tobago	
H. ocillum	tropics	Bogdan, 1977
	Malaysia	Lenné, 1990
Leptosphaeria trifolii (Rostrup) Petrak	Trinidad	Lenné, 1990
L. taiwanensis Yen & Chi	Taiwan	Leu *et al.*, 1974
Magnaporthe grisea	Colombia	Lenné, 1990
	Papua New Guinea	
	Puerto Rico, Tanzania	
	Venezuela	
Nigrospora Zimm. sp.	Cambodia	Lenné, 1990
N. sphaerica (Sacc.) Mason	Malaysia	Lenné, 1990
Phoma sp.	not given	Lenné & Trutmann, 1994
Phoma sorghina (Sacc.) Boer., Dorenb & van Kest.	Malaysia	Lenné, 1990
P. pennisetina Zimm.	India	Lenné, 1990
Phyllosticta sp.	USA	Lenné, 1990
Puccinia chaetochloae Arthur	Mauritius	Lenné, 1990
P. substriata var. *penicillariae* Ramachar & Cumm.	Venezuela	Lenné, 1990
P. stenotaphri Cumm.	USA	Lenné, 1990
Pyricularia Sacc. sp.	Colombia, Malaysia	Lenné, 1990
Pyricularia didyma M. B. Ellis	Cuba, Venezuela	Lenné, 1990
Pyricularia (=*Magnaporthe*) *grisea*	Venezuela	Malaguti *et al.*, 1972
Sclerophthora macrospora Sacc.	Australia	Lenné, 1990
Sclerospora graminicola (Sacc.) Schröt.	India	Lenné, 1990
S. macrospora Sacc.	not given	Lenné, 1990

Table 6. Records of bacteria, viruses, nematodes, and diseases of unknown cause, associated with *Pennisetum purpureum*.

problem	country	reference
bacteria		
Xanthomonas albilineans (Ashby) Dowson	Cameroon	Rott *et al.*, 1988
viruses		
maize mosaic (stripe disease)	India	Chatterjee & Nimbalkar, 1977
maize streak geminivirus[1]	not given	Brunt *et al.*, 1990
geminivirus	Zimbabwe	Rose, 1973
potyvirus	Brazil	Martins & Kitajima, 1993;
	Ethiopia	J Hanson (*pers. comm.*)
sugarcane chlorotic streak	Taiwan	Anon., 1977
sugarcane mosaic	India	Rishi *et al.*, 1973
disease of unknown cause		
stunting	Uganda	Tiley, 1969[2]
nematodes		
Belonolaimus longicaudatus Rau (Tylenchida:Belonolaimidae)	USA	McSorley *et al.*, 1989
Bitylenchus (= *Tylenchorhynchus*) *vulgaris* Upadhay, Swarup & Sethi (Tylenchida:Dolichodoridae)	India	Vaishnav & Sethi, 1977
Hemicycliophora corbetti Siddiqi (Tylenchida:Hemicycliophoridae)	Malawi	Siddiqi, 1980
Macroposthonia (= *Criconemella*) *ornata* (Raski) De Grisse & Loof (Tylenchida:Criconematidae)	USA	McSorley *et al.*, 1989
M. sphaerocephala (Taylor) De Grisse & Loof	USA	McSorley *et al.*,1989
Malenchus tantalus Siddiqi (Tylenchida:Tylenchidae)	Malawi	Siddiqi, 1979
Meloidogyne hapla Chitwood (Tylenchida:Meloidogynidae)	Peru	Vargas & Pajuelo, 1973
M. incognita Chitwood	India	Vaishnav & Sethi, 1977
	West Africa	Luc & de Guiran, 1960
	Peru	Vargas & Pajuelo, 1973
M. javanica (Treub) Chitwood	Rhodesia	Martin, 1956
M. (= *Heterodera*) *marioni* species inquirendae	USA	Godfrey, 1935
Neomalenchus malawiensis Siddiqi (Tylenchida:Tylenchidae)	Malawi	Siddiqi, 1979
Pratylenchus Micoletzky sp. (Tylenchida: Pratylenchidae)	India	Anon., 1985
P. brachyurus (Godfrey) Goodey	West Africa	Luc & de Guiran, 1960
Radophilus similis (Tylenchida:Pratylenchidae)	India	Anon., 1974b
Varotylus (= *Orientylus*) *varus* (Jairajpuri & Siddiqi) Siddiqi (Tylenchida:Hoplolaimidae)	Malawi	Jairajpuri & Siddiqi, 1977
Xiphinema elongatum Sch., Stekhoven & Teun. (Dorylaimida:Londidoridae)	China	Fang, 1994
X. imitator Heyns		
X. insigne Loos		
X. radicola Goodey		
Zanenchus zanclus Siddiqi (Tylenchida:Tylenchidae)	Malawi	Siddiqi, 1979

[1] In a survey of 17000 Napier grass stools in Kenya, Njuguna *et al.* (1997) found no evidence of MSV infection and contended that this virus does not infect the grass.
[2] Tiley suggested an insect borne virus as the cause.

2.1.5 *Pennisetum purpureum* as a weed

Although it is usually grown as a crop, Napier grass is also regarded as a weed in some instances. The grass was reported as a weed in sugarcane in Hawaii, USA (Sylvester, 1990), in vineyards and citrus in Israel (Bahat, 1985), in sisal in Mozambique (Hindorf, 1972), as a general weed in Thailand (Harada *et al.*, 1991) and in Florida, USA on ditchbanks by Orsenigo (1977). In Nigeria, Michieka and Akobundu (1984) evaluated herbicides for its control in no-tillage maize.

2.2 The pathogen cohort - smut fungi

Smut fungi are a major group of plant pathogens and parasites. It is likely they have been of importance on food crops since early times, because cultivated crops were originally derived from wild ancestors which are known to suffer from diseases that are related to smut. However, it is difficult to separate early records of smut infection from descriptions of other fungal diseases, as the terms *blast*, *blight*, *rust* and *smut* were used interchangeably. Fischer and Holton (1957) suggested that smuts were recognised by the ancient Romans and Greeks. Tillet (1755) made the original classic contribution to the scientific elucidation of smuts as plant parasitic fungi, with his work on bunt or stinking smut of wheat, and one of the earliest descriptions of the gross effects of smut diseases was given by Tozzetti (1767).

Cereal smuts were of particular economic importance historically, because they

were responsible for serious losses in wheat, oats, barley, sorghum and maize worldwide. Infection by such smuts, which attack and destroy the inflorescence, can lead to complete loss of the seed head. Up to the Second World War, periodic losses attributed to smuts (of 30-40 percent) were recorded in the major cereal growing areas of western USA, Canada, India and Australia (Fischer and Holton, 1957). These cereal smuts have since largely been controlled by seed dressings and resistant varieties, though the 1996 outbreak of Karnal bunt (caused by *Tilletia* (= *Neovossia*) *indica* Mitra) in the USA *"...will have a significant impact on the US farm economy"* (APS, 1997). Other smuts, such as that of sugarcane caused by *Ustilago scitaminea* Sydow, are usually of sporadic or minor importance, with occasional devastating outbreaks. Ornamentals such as carnations, and vegetables such as onions are also attacked. In addition to direct losses, smut spores can have adverse effects on human and animal health. Fischer and Holton (1957) reported a number of cases of respiratory allergenic effects in those handling smutted grains, either at harvest or during milling. They also noted acrodynia (a disease in which reddening of the hands and feet are the major symptoms) and dermatitis. Fischer and Holton (1957) reviewed the evidence of the effects of smut infested produce when fed to animals, but concluded that there was no clear cut proof of adverse reactions.

There is little mention of positive uses for smuts, though Terrell and Batra (1982) gave details of a smut-plant association between Manchurian wild rice

(*Zizania latifolia* Turez) and *U. esculenta* Hennings. The fungus causes enlarged culms which have been used in China as a vegetable (*gau sun*) since the 10th century. Smutted heads of maize, *huitlacoche* or *cuiclacoche*, are regarded as delicacies in Mexican cuisine (Kennedy, 1978). Recently, attention has focussed on the effects of smut diseases on grass weeds, to assess their potential as bio-control agents (Ellison and Evans, 1990), and Antonovics (1998) has proposed the study of anther smut disease of *Silene* sp. as a model system for the elucidation of the epidemiology of animal and human sexually transmitted diseases.

2.2.1 Morphology of smut fungi and disease symptoms

Morphologically, the smut fungi comprise a comparatively simple group (Fischer and Holton,1957). The two basic elements are the vegetative mycelium and the reproductive structures, ie the spores and their associated forms. The mycelium is parasitic, though many species have shorter or longer saprophytic life cycles (Vánky, 1987). Growth is intercellular within the host plant, and the fungus may not reveal its presence until sporulation begins. In some cases, hyperplasia or hypertrophy may result from smut infection. Although smut infections are usually systemic, sporulation is characteristically restricted to specific parts of the host, such as stems (typically on grasses; *Ustilago hypodytes* (Schlechtendal) Fries, *U. grandis* Fries, *U. jacksonii* Zundel and Dunlap, *Urocystis fraseri* Clinton and Zundel and others), leaves (on grasses and cereals; *Ustilago striiformis* (Westendorp) Niessl and *U. longissima*

(Schlechtendal) Meyen), inflorescences (on cereals; *Ustilago nuda* (Jensen) Rostrup and *U. avenae* (Persoon) Rostrup) and roots (*Urocystis coralloides* Rostrup on some Cruciferae). Galls may also be formed, for example *Ustilago maydis* (De Candolle) Corda on maize and *U. crus-galli* Tracy and Earl on *Echinochloa* Beauvois spp. In the latter case the galls are not restricted to any one site.

Smut fungi can absorb nutrients from the host through lobed haustoria (as in the rust and powdery mildew fungi) or directly through the cell walls.

If the host is perennial then the mycelium may also be perennial, continuously producing spores in a fruiting structure called a sorus (the sorus is composed of fungal and host tissues). Indeed, the immediate, and usually most obvious, sign of smut infection on host plants is the presence of black, spore bearing sori. However, there may be secondary effects, which Fischer and Holton (1957) divide into two main categories: morphological and physiological. Table 7 provides a summary of these secondary effects.

Table 7. Secondary effects of smut infection on host plants (from Fischer and Holton, 1957).

effect	smut pathogen	host
morphological modifications		
hemaphroditism-stamens formed on female flowers of dioecious plants	*Ustilago violacea* (Pers.) Roussel	Caryophyllaceae
heterostyly-styles longer than anthers	*Ustilago oxalidis* Ellis and Tracy	*Oxalis europeae* Jordan
dehiscence-pericarp dehisces to facilitate dissemination of smut spores	*Ustilago dehiscens* Ling	*Polygonum amplexicaule* D. Don.
production of vestigial ovaries-these develop into smut balls	*Tilletia buchloeana* Kellerman and Swingle *Sorosporium everhartii* Ellis and Galloway	*Buchloë dactyloides* Engelmann *Andropogon furcatus* H. L. Muehlenb.
alliophylly-enlarged leaf blades and flowers	*Urocystis anemone* (Pers.) Winter	*Anemone nemorosa* L.
phyllody-proliferation of flower parts	*Ustilago bullata* Berkeley *Ustilago maydis*	*Bromus erectus* Hudson *Zea mays* L.
inflorescence modifications	*Tilletia* Tul & C. Tul. spp.	*Triticum* L. spp.
dwarfing and induced tillering	*T. foetida* (Wallroth) Liro *T. caries* (De Candolle) Tulasne	*Triticum* spp.
root reduction	*Ustilago nuda*	*Triticum* spp.
seedling deformation and leaf spotting	*Urocystis tritici* Körnicke *Ustilago avenae*	*Triticum* spp. *Triticum* spp.
sterile heads-a result of latent infection	*Ustilago trachypogonis* Zundel	*Triticum* spp.
physiological modifications		
increased susceptibility to rusts	*T. caries*	*Triticum* spp.
increased susceptibility to seedling blight and root rot	*T. caries*	*Triticum* spp.
increased susceptibility to powdery mildew	*T. caries*	*Triticum* spp.
increased susceptibility to winter injury	*T. caries* *Ustilago avenae*	*Triticum* spp.
reduced sugar levels	*U. maydis*	*Zea mays*

2.2.2 Taxonomy of smut fungi

Smuts and rusts are included in the subdivision Basidiomycotina of the class Ustomycetes (Hawksworth *et al.*, 1995). Fungi in this class are characterised by the production of thick walled, bi-nucleate spores; teliospores in rusts and ustilospores in smuts. However, rusts have sex organs (spermatia and receptive hyphae) whereas in smuts, the dikaryon can be formed from the fusion of any vegetative part (Alexopoulos and Mims, 1979). Smut fungi are in the order Ustilaginales, the name being derived from the predominant, very dark (almost black in mass) colour of the spores (Holliday, 1989). The Ustilaginales have long been regarded as a distinct group (Hawksworth *et al.*, 1995).

The classification of the smut fungi is not fully agreed and remains open to interpretation. Their taxonomic status has been repeatedly subject to change, ever since the first attempt at classification by Persoon (1801). Much debate concerns the number of smut families, and the criteria for discrimination between them. The following discussion on smut classification is taken largely from Fischer and Holton (1957), Mordue and Ainsworth (1984), Vánky (1987) and Hawksworth *et al.* (1995).

Historically, smut fungi have been differentiated on the basis of ustilospore germination products. Based on the morphology of the promycelium and location of the basidiospores, Tulasne and Tulasne (1847) divided the

Ustilaginales into two families, the Ustilaginaceae and the Tilletiaceae. Brefeld (1885) proposed the term Hemibasidiomycetes for the class that contained the Ustilaginales.

A third family, the Yeniaceae (*nomen nudum*), was erected by Liou (1949), justified by an intermediate type of ustilospore germination. Ciferri (1963) discounted the Yeniaceae, but identified four other families, again based on germination type: Entylomellaceae (for the anamorphs), Tilletiaceae, Glomosporiaceae and Ustilaginaceae. Recent discoveries of yeasts and saprophytic fungi, with heterobasidiomycetous life cycles very similar to that of smut fungi, led to the proposal of the class Endomycetes by von Arx (1967) and the family Filobasidiaceae by Olive (1968). Cox (1976) supported Filobasidiaceae but moved it to the order Aphyllophorales in the class Homobasidiomycetes, whereas Moore (1978) disagreed with this contention and placed it in the order Tremellales, class Heterobasidiomycetes.

The family Graphiolaceae (which parasitize palms) has been included by some taxonomists within the Ustilaginales, such as Oberwinkler *et al.* (1982). They classified the Graphiolaceae as basidiomycetes in the new order Graphiolales Donk ex Oberwinkler & Bandoni (Vánky, 1987). Although species of this family lack the thick walled ustilospore normal in the Ustilaginales, recent molecular data suggest the Graphiolaceae are closely allied to the smuts (Hawksworth *et al.*, 1995).

Anomalies with smut classification were recognized at the beginning of the century. Cunningham (1924) pointed out that some smut fungi did not produce a promycelium from germinating ustilospores. A further complication was that spore germination in many species had not been observed, and it was known that the type of germination could be influenced by environmental factors such as temperature, humidity, pH and presence or absence of different substances. For these reasons, Cunningham (1924), Fischer (1953) and Lindeberg (1959) rejected the division into families and considered all smuts to belong to one family, the Ustilaginaceae. However, Zambettakis (1967) and Alexopoulos and Mims (1979) discounted these concerns and maintained that the families Tilletiaceae and Ustilaginaceae were sufficiently different to warrant continued use. Watson (1972) agreed with Zambettakis (1967), and pointed out that the processes of classification and identification were distinct. The fact that the criteria were not practical for identification purposes was not in itself a valid argument for abandoning the families.

Ainsworth (1973) proposed the systematic placing of the smut fungi to be: Kingdom Mycetaceae, Division Amastigomycota, Subdivision Basidiomycotina, Class Teliomycetes, Order Ustilaginales. A more recent classification was given by Holliday (1989) as Division Eumycota, Subdivision Basidiomycotina, Class Hemibasidiomycetes, Order Ustilaginales, Family Ustilaginaceae. Hawksworth *et al.* (1995) retained the Class Basidiomycetes as being *"...one of the [sub] divisions of much of the literature dealing with these [smut] fungi"*.

Moore (1996) proposed a new taxonomic synopsis for the ten orders of the Ustomycota: those producing ustospores (which includes the Ustilaginales), those producing ustidiomes (the Agaricostilbales and three others) and the Exobasidiales that form neither. He also divided the order Tilletiales between the Tilletiaceae and the Doassansiaceae. The Ustilaginales were split into the Ustilaginaceae (emended) for species on monocots and the Microbotryaceae (*fam. nov.*) for species on dicots.

Hawksworth *et al.* (1995) described the current concept of Ustomycetes as *".. broad... with the smuts divided into two families"*. They pointed out that the characters of the promycelium were sufficiently distinct to assign possible ordinal ranks, although elevation required additional support from molecular and ultrastructural data at present available for only a few species.

Bauer *et al.* (1997) used characteristics of hyphal septation and zones of host-parasite interaction to support the hypothesis of two phylogenetically separate lines of smut fungi. The first line is the Microbotryales (two families and six genera). The second monophyletic line is the Ustilaginomycetes, comprising three lineages (one of which, the Ustilaginomycetidae, contains the genus *Ustilago*).

Bisby and Ainsworth (1943) listed 34 genera that could be distinguished with certainty in morphological terms (with an additional 29 of uncertain attribution),

comprising 700 species. Fischer and Holton (1957) gave 33 genera and 1162 species, but recognised that some were "...*on a rather shaky foundation.*", such as *Tolyposporium* Woronin, *Glomosporium* Kochman and *Thecaphora* Fingerhuth. According to Duran (1973), there are approximately 1100 smut species, whereas Vánky (1987) estimated the number of known species at about 1200, distributed over 51 genera with 3000 synonyms for the species and 30 for the genera. Hawksworth *et al.* (1995) refer to the Ustilaginaceae as comprising 33 genera (with 19 synonyms) and 629 species. The taxonomic flux has continued, with Moore (1996), Vánky (1991, 1992, 1993, 1994, 1995a, 1995b, 1996a, 1996b, 1997), Websdane *et al.* (1994), Piepenbring *et al.* (1996) and Shivas and Vánky (1997) describing new species or reclassifying existing species. Vánky (1987) asserted that the different numbers of species, as given by various authors, depends on the definition of the term *species*, rather than reflecting an absolute accounting.

2.2.3 Host range of smut fungi

With regard to host range, Fischer and Holton (1957) listed 77 plant families from which smuts had been identified. All but three were found on Angiospermae (the exceptions are *Ustilago fussii* Niessl on *Juniperus nana* L. and *Melanotaenium oreophilum* Sydow and *M. selaginellae* Hennings and Nyman on *Selaginalla* Beauvois spp.). Most were reported from the Gramineae (623 species in 14 genera), Cyperaceae (111 species in 16 genera), Compositae (63 species in four genera) and Polygonaceae (54 species in six

genera). Table 8 shows smuts of economically important crops worldwide.

Four genera, *Ustilago* (Persoon) Roussel, *Sorosporium* Rudolphi, *Sphacelotheca* de Bary and *Tilletia*, comprise over 90 percent of smuts on grasses (Fischer and Holton, 1957). Lenné (1990) listed 90 species in 23 smut genera on tropical pasture grasses in Africa. However, references to smuts on wild grasses in Africa are scarce. Dennis (1988) examined 239 sheets of the grass *Sporobolus spicatus* (Vahl.) Kunth. at the Royal Botanic Gardens, Kew, and found *Melanotaenium majus* (Hariot and Patouillard) Ciferri on three specimens, from Sudan, Zaire and Kenya. The type species was from Chad. Dennis also proposed a new variety, *M. majus* var. *melinidis*, on *Melinis macrochaeta* Stapf & Hubbard from Nigeria.

Table 8. Smuts of economically important graminaceous crops worldwide.

pathogen	common name	host	reference
T. controversa Kühn in Rabenh.	dwarf bunt	barley, *Hordeum vulgare* L. amend. Bowden	Mathre, 1993
Ustilago hordei (Pers.) Lagerh.	covered smut		
U. tritici (Pers.) Rostr. = *U. nuda*	loose smut		
U. avenae = *U. nigra* Tapke	semi-loose smut		
Ustilago zeae (Beckm.) Unger = *U. maydis*	common smut	maize, *Zea mays*	Shurtleff *et al.*, 1993
Sphacelotheca reiliana (Kühn) Clinton = *Sporisorium holci-sorghii* (Rivolta) Vánky	head smut		
Ustilago segetum (Bull.:Pers.) Roussel = *U. kolleri* Willie	covered smut	oats, *Avena sativa* L.	Epstein and Simons,1993
U. avenae	loose smut		
Moesziomyces penicillariae (Bref.) Vánky = *Tolyposporium penicillariae*	smut	pearl millet, *Pennisetum glaucum* (L.) R. Br.	Wilson and Claflin, 1996
Neovossia horrida (Takah.) Pad. & Khan	kernel smut	rice, *Oryza sativa* L.	Hollier *et al.*, 1993
Sacc. & Syd. in Sacc.			
Entyloma oryzae Syd. & Syd.	leaf smut		
Tilletia caries = *T. tritici* (Bjerk.) Wint. in Rab.	bunt, stinking smut	rye, *Secale cereale* L.	Cunfer *et al.*, 1993
T. laevis Kühn in Rabenh. = *T. foetida*			
T. controversa	dwarf bunt		
Neovossia indica = *T. indica*	Karnal bunt, partial bunt		
Ustilago tritici	loose smut		
Urocystis occulta (Wallr.) Rab. ex Fuckel	stalk smut		
Sporisorium sorghi Link in Willd.	covered kernel smut	sorghum, *S. bicolor* (L.) Moench	Horne and Frederiksen, 1993
Sphacelotheca reiliana = *Sporisorium holci-sorghii*	head smut		
Sporisorium cruentum (Kühn) Vánky = *Sphacelotheca cruenta* (Kühn) Potter	loose kernel smut		
Ustilago scitaminea	culmicolous smut	sugarcane, *Saccharum* L. spp.	Ferreira and Comstock, 1993
Entyloma dactylidis (Pass.) Cif.	blister smut	turf grasses, A, Ag, F, L, P	Smiley, 1993
Tilletia caries	covered smut	Ag, P	
T. controversa		Ag, L, P	
T. laevis		Ag, L, P	
Urocystis agropyri (Preuss) Schröt.	flag smut	A, Ag, F, L, P	
T. lolliі Auers.	loose smut	L	
Ustilago affinis Ellis & Everh.		S	
U. bullata		Ag	
U. cynodontis (Henn.) Henn.		C	
U. hypodytes	sheath smut	Ag	
U. striiformis	stripe smut	A, Ag, F, L, P	
Tilletia caries	common bunt, stinking smut	wheat, *Triticum* L. spp. L.	Weise *et al.*, 1993
T. controversa	dwarf bunt		
Urocystis agropyri	flag smut		
Neovossia indica	Karnal bunt, partial bunt		
Ustilago tritici	loose smut		

A = *Agrostis canina* L., *A. palustris* Huds. and *A. tenuishn* Sibth.; Ag = *Agropyron cristatum* (L.) Gaertn.; C = *Cynodon dactylon* (L.) Pers. or *Cynodon* Rich. hybrids; F = *Festuca arundinacea* Schreb., *F. longifolia* Thuill., *F. ovina* subsp. *glauca* L., *F. rubra* subsp. *commutata* Gaud. and *F. rubra* subsp. *rubra* L.; L = *Lolium multiflorum* Lam. and *L. perenne* L.; P = *Poa annua* L., *P. pratensis* L. and *P. trivialis* L.; S = *Stenotaphrum secundatum* (Walt.) Kuntze.

2.2.4 Smut diseases in Kenya

Four smut genera have been described on cereals and sugarcane in Kenya (*Sphacelotheca, Tilletia, Tolyposporium* and *Ustilago*). Tables 9 and 10 show the most up to date records of smuts in the country.

Most smuts are pathogens of land dwelling hosts, though *Burrillia* Setchell, *Doassansia* Cornu, *Doassaniopsis* (Setchell) Dietel, *Nannfeldtiomyces* Vánky, *Narasimhania* Thirumalacher & Pavgi, *Pseudodoassania* (Setchell) Vánky, *Rhamphospora* D Cunningham and *Tracya* Setchell are found on aquatic or paludal plants (Vánky, 1987).

2.2.5 Generalised life cycle of the smut fungi

Smut fungi have a simpler life cycle than rusts, but unlike rusts cause extensive systemic invasion before sporulation develops. The soma of a smut fungus consists of haploid cells and dikaryotic mycelium. In some species the haploid form is capable of prolonged yeast-like reproduction on non-living strata. The dikaryotic form is usually obligate, though it is now recognised that the dividing line between saprophytic and obligate forms may not be sharply delineated (Vánky,1987). The mycelium is branched, usually intercellularly, often with haustoria extending within the host cells. In most cases it is asymptomatic or even dormant, and gives little evidence of its presence until spore formation begins. The mycelium may be more or less systemic, or localised in various parts or organs of the host.

Table 9. Smuts of graminaceous crops in Kenya.

smut	common name	host	date/reference
Sphacelotheca (=*Sporisorium*) (Kuehn) Potter	loose smut	sorghum, *Sorghum sudanense* Stapf	1939 in Anon., unpub.(a); Nattrass, 1961
S. (=*Sporisorium*) *sorghi* Clinton	covered kernel smut	*Sorghum caudatum* Stapf *S. bicolor*	1924 in Anon., unpub.(a); McDonald, 1936
S. reiliana (= *Sorosporium reilianum* (Kuehn) McAlpine)	head smut	*S. caudatum* *S. sudanense* *S. bicolor* *Zea mays*	Duke, 1926
Tilletia foetida	stinking smut	*Triticum vulgare* Host.	1953 in Anon., unpub.(a); Nattrass, 1961
Tolyposporium (=*Sorosporium*) *ehrenbergii* (Kühn) Ling	long smut	*S. bicolor*	Julian, 1995
T. penicillariae		millet, *Pennisetum typhoides*	Duke, 1926
Ustilago avenae		oat, *Avena sativa*	1934 in Anon., unpub.(a); McDonald, 1936
U. hordei		barley, *Hordeum vulgare*	1928 in Anon., unpub.(a); McDonald,1936
U. maydis	common smut	maize, *Zea mays*	Gatumbi, 1985
U. nuda (=*U. tritici* Jensen)		*H. vulgare* and wheat, *Triticum vulgare*	1929 in Anon., unpub.(a); McDonald, 1936
U. scitaminea	sugarcane smut	sugarcane, *Saccharum officinarum* L.	1958 in Anon., unpub.(a); Robinson, 1959

Table 10. Smuts of lesser economic importance in Kenya.

smut fungus	host	reference
Cintractia axicola (Berkeley) Cornu var. *minor* Clinton	*Cyperus* L. sp.	McDonald, 1936
**Cintractia dubiosa*	*Pennisetum* sp.	McDonald, 1936
C. limitata Clinton	*Cyperus rotundus* L.	Ondieki, 1973
Doassansiopsis nymphaeae (Sydow) Thirumalacher (=*Doassansia nymphaeae* (Sydow)) Thunberg	*Nymphaea zanzibariensis* Casp. (=*N. capensis*)	Nattrass, 1961
Entyloma de Bary	*Panicum* L. sp.	Ondieki, 1973
E. australe Spegazzini	*Physalis peruviana* L.	Nattrass, 1961
E. bidentis Hennings	*Bidens pilosa* L.	1948 in Anon., unpub.(a); Nattrass, 1961
E. calendulae (Oudemans) de Bary	*Calendula officinalis* L.	Nattrass, 1961
E. dahliae Sydow	cultivated *Dahlia* Cav.	1938 in Anon., unpub.(a); Nattrass, 1961
E. physalidis (Kalchbr. & Cooke) Wint.	*Physalis peruviana*	1949 in Anon., unpub.(a);
E. zinniae Sydow	*Zinnia pauciflora* L.	Nattrass, 1961
Melanopsichum pennsylvanicum Hirschhorn	*Polygonum senegalensis* Meisner	1950 in Anon., unpub.(a); Nattrass, 1961
	P. setulosum Rich.	Anon., unpub.(b)
Melanotaenium majus	*Sporobolus spicatus*	Dennis, 1988
Sorosporium holstii Hennings	*Themeda triandra* Forsk.	Nattrass, 1961
S. penniseti Mundkur	*Cenchrus ciliaris* L.	Ondieki, 1973
Sphacelotheca kenyae Zundel	*Hyparrhenia* Anders. ex. Fourn.	Lenné, 1990
S. ophiuri (Hennings) Ling	*Rottboellia exaltata* L.	Nattrass, 1961
S. themedae Duke	*Themeda triandra*	1933 in Anon., unpub.(a)
Tilletia sp.	*Entolasia imbricata* Stapf and *Eragrostis caespitosa* Chiov.	Nattrass, 1961
T. ayresii Berkley ex Massee	*Panicum maximum* Jacq.	Duke, 1926
T. echinosperma Ainsworth	*Setaria* Pal. spp.	Bogdan, 1955
	Setaria longiseta Beauv.	1957 in Anon., unpub.(a)
	S. splendida Stapf (=*S. aurea* Dur. & Schum.)	1956 in Anon., unpub.(a)
	S. sphacelata (Schumacher) Stapf & Hubbard ex Moss	Robinson, 1960
	S. trinervia Stapf	1958 in Anon., unpub.(a)
T. vittata (Berkeley) Mundkur	*Oplismenus compositus* (L.) Beauv.	Ondieki, 1973
Ustilago sp.	*Schmidtia bulbosa* Stapf	Nattrass, 1961
	Schmidtia pappophoroides Stend.	Anon., unpub.(b)
	Dactyloctenium Willd.	Ondieki, 1973
U. (=*U. bullata*) *bromivora* (Tul.) Fischer de Waldheim	*Bromus ?unioloides* HBK	1957 in Anon., unpub.(a)
U. bullata	*B. unioloides*	Nattrass, 1961
U. crameri Körnicke	*Setaria italica* Beauvois	McDonald, 1936
U. cynodontis	*Cynodon plectostachyum* (Schumacher) Pilger	1951 in Anon., unpub.(a)
	C. dactylon	Nattrass, 1961
U. goniospora Massee	*Aristida* L. sp.	Duke, 1926
U. hitchkockiana Zundel	*C. dactylon*	Nattrass, 1961
U. heterospora Henn. (= *U. evansii* Henn.)	*Setaria splendida*	1930 in Anon., unpub.(a)
U. scheffleri Sydow	*Pennisetum clandestinum* Hochst. ex Chiov. (= *P. inclusum* Pilger)	Sydow & Sydow, 1910
U. syntherismae (Schweinitz) Peck (=*U. rabenhorstiana* Kuehn)	*Digitaria* Hall sp.	1938 in Anon., unpub.(a); Nattrass, 1961
	D. horizontalis Stapf	1949 in Anon., unpub.(a)
	D. velutina (Fors.) Beauv.	Nattrass, 1961
U. utriculosa (Nees) Unger	*Polygonum* L. sp.	1943 in Anon., unpub.(a)
	P. senegalense Meisn.	Gatumbi, 1985

*This is unlikely to be a *Cintractia* species since Fischer and Holton (1957) attest that this genus does not occur on the Graminae. Vánky (1987) concurred, stating that the ten species of *Cintractia* are found only on the Cyperaceae.

The infection cycle begins when fungal propagules penetrate the surface of the host plant. This may be at the seedling stage (penetration from seed-borne spores, seed-borne mycelium or by soil-borne spores or mycelium); through the embryo (also known as blossom or flower infection - as in *Ustilago hordei*); through the shoot (as in *U. scitaminea* on sugarcane) and through local infection, whereby infection is restricted to the organ or region of the plant initially penetrated (as in *U. maydis* on maize).

The time between infection and sporulation is the incubation period, and varies considerably in different species. In *U. maydis* the incubation period may be as short as ten days, whereas in stem smut of grasses, *U. spegazzini* Hirschhorn, two or three years may elapse before sporulation (Fischer, 1945).

Smut spores (formerly called chlamydospores, teliospores or ustospores but now more usually known as ustilospores), are the means of dispersal and survival outside the host. They are formed in sori which consist of host tissues, spore masses and possibly also of modified fungal tissue. Sori are usually visible and may be locular, embedded in the host tissue (*Angiosorus* Thirumalacher and O'Brien), on leaves (*Burrillia*), in galls on roots (*Entorrhiza* C Weber), on anthers (*Microbotryum* Léveillé), on fruits and stems (*Mundkurella* Thirumalacher) and on spikelets and ovaries (*Ustilago* and *Tilletia*). The sori of *Schroeteria* Winter, which infests *Veronica* L. spp., are cryptic, such that infected plants do not differ outwardly from healthy ones.

The spores may be borne singly, in pairs or clumped in groups as spore balls. Spore balls may be entirely composed of fertile spores or a combination of fertile spores, sterile cells and/or hyphae. Spores are usually dark brown in colour, powdery *en masse*, with thick walls which may be smooth or sculptured (Holliday,1989). Spore size varies from 3.5μm (in *Ustilago minima* Arthur and *U. trebouxii* Sydow) to 50μm (in *Tilletia paradoxa* Jaczewski) (Fischer and Holton,1957). Dispersal is mainly by wind, with insects, man and water contributing in a minor way.

Generally, though there are exceptions, germinating diploid spores give rise to a promycelium (a basidium or ustidium) in which meiosis occurs to produce haploid sporidia (basidiospores), or, more rarely, haploid hyphae. The haploid forms fuse in pairs to restore the dikaryophase. Some species may posses an anamorph, or conidial, state (Vánky, 1987). Figure 3 is a schematic representation of the nuclear phases of the smut life cycle.

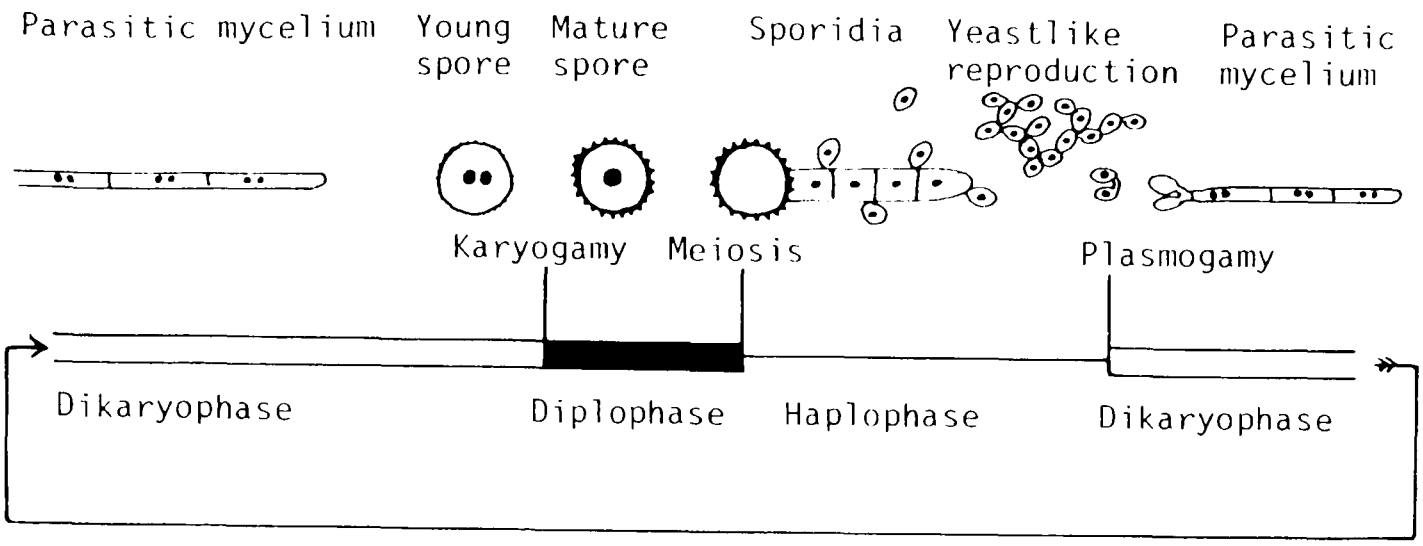


Figure 3. Generalised scheme of nuclear phases of smut fungi (from Vánky, 1987).

The colour, size, shape and ornamentation of the spores and sterile cells, the structure of the spore balls, and sori and gemination type vary considerably and are important characters in the taxonomy of the Ustilaginales, particularly at species level (Mordue and Ainsworth,1984).

2.2.6 The genus *Ustilago*

As for the family as a whole, the taxonomy of *Ustilago* has been subject to much revision. The genus was first described by Persoon in 1801 as *Uredo,* subgenus *Ustilago*. Roussel (1806) transferred it to *Ustilago.* Ciferri (1953) proposed a division of the genus (including *Sorosporium*) into the subgenera of *Ustustilago, Ustosporium, Ustisorotheca, Ustisoractia* and *Ustisorcintheca.* The problems of delineation were discussed by Lindeberg (1959).

The criteria for the genus *Ustilago* were more recently given by Langdon and

Fullerton (1975), based on the absence of collumellae and peridia. Fischer and Holton (1957) described 331 *Ustilago* species, though Vánky (1987) reported more than 350. Hawksworth *et al.* (1995) describe the genus as "... *biologically distinct but morphologically close and variously treated as species, varieties or even physiologic races.*"

Ustilago sori are found in various parts of the host. At maturity they burst to expose a powdery or agglutinated blackish-brown, purplish-brown, olivaceous, or more rarely pale, spore mass. There are no sterile cells. The spores are single, small to medium sized (rarely over 20μm in diameter), with smooth verruculose, echinate or reticulate walls. Spore germination is by means of a septate promycelium bearing sporidia or infection hyphae produced terminally or laterally (Vánky, 1987). Of all the smut genera, *Ustilago* contains the most species, parasitizing a large number of host types. The genus is heterogeneous and its limits, compared with *Sorosporium*, *Sphacelotheca*, *Sporisorium* Ehrenberg ex Link and *Microbotryum*, are not always clearly marked (Vánky, 1987).

2.2.7 Hosts of *Ustilago* species

The genus *Ustilago* infects at least 27 genera of flowering plants, though most *Ustilago* species are found on the Gramineae (227 grass species), with 36 species of the Liliaceae and 12 species of the Polygonaceae reported infected. *Ustilago* species comprise 36 percent of those smuts that parasitize grasses (Fischer and Holton,1957). Historically, most reports have concerned smut

occurrence on wild grasses, but Table 11 gives some recent records of *Ustilago* species on grasses of economic use. Only one species of *Ustilago* infects a gymnosperm, the rest are found on angiosperms.

2.3 *Ustilago kamerunensis* H Sydow and Sydow

This species was first described by Sydow and Sydow in 1910, from specimens of *Pennisetum purpureum* Schumacher collected from Cameroon (hence the specific name *kamerunensis*). Ovaries and inflorescences were attacked, with the inflorescences completely destroyed. The sori transformed the inflorescence into a mass of brown, powdery spores, leaving a naked rachis. The ustilospores were spherical, clear reddish-brown, smooth and regular. Spore diameter was 6-8μm (Zundel, 1953; Zambettakis, 1970). Figure 4 shows Zambettakis' (1970) drawings of teliospores and a smutted inflorescence.

Ustilago kamerunensis has not been reported beyond Africa. Table 12 shows country records of first occurrences to date. Appendix 1 gives the original description by Sydow and Sydow in 1910 (in Latin), a later rewording of the Sydow and Sydow text by Zundel (1953) and a French description by Zambettakis in 1970, derived from these two earlier references.

Table 11. *Ustilago* species on economically valuable grasses.

pathogen	grass	use	country	reference
Ustilago bullata *U. bullata* var. *macrospora* (Farlow) Fischer	brome grass *Bromus* Dill ex L. sp.	pasture	Argentina	Hirschhorn, 1973
U. bullata	prairie grass *B. willdenowii* Kunth.	pasture	New Zealand	Falloon *et al.*, 1988
	mountain brome, *B. sitchensis* Trin.	pasture	New Zealand	Falloon *et al.*, 1988
	B. stamineus Desv.	pasture	New Zealand	Stewart, 1992
U. destruens Hausmann	fall panicum, *Panicum dichotomiflorum* Michx.	weed	Canada	Govinthasamy & Cavers, 1995
U. scitaminea	Munj grass, *Saccharum munja* Roxb.	fodder, roofing	India	Rao *et al.*, 1990
U. striiformis	salt marsh grass, *Puccinellia maritima* (Hudson) Parl.	pasture	Outer Hebrides UK	Spooner, 1991
	Kentucky blue grass, *Poa pratensis*		USA	Turgeon *et al.*, 1974
	red fescue, *Festuca rubra*		USA	Turgeon *et al.*, 1974

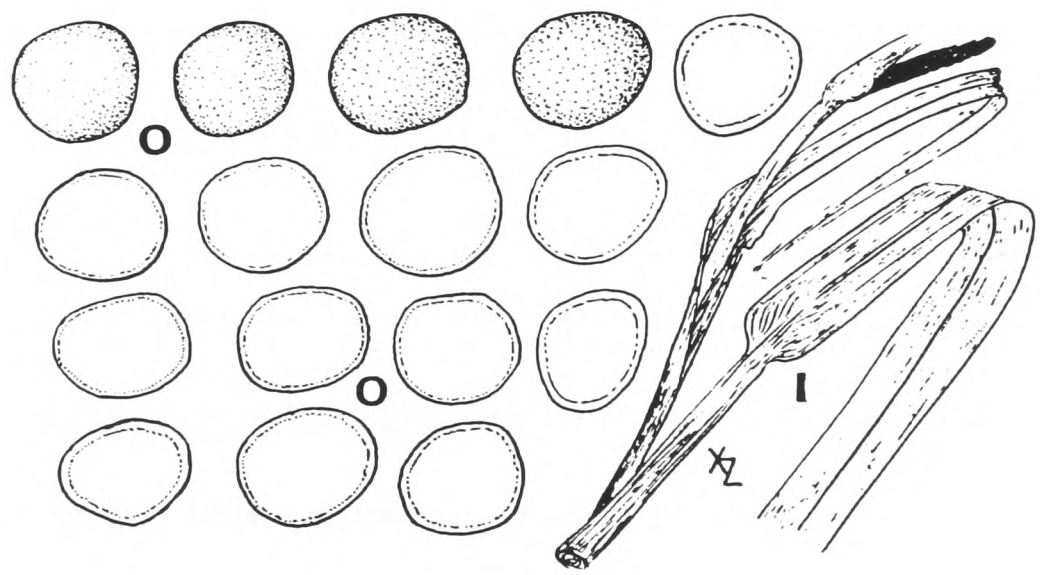


Figure 4. *Ustilago kamerunensis* H Sydow & Sydow on *Pennisetum* sp. I - infested inflorescence; O - ustilospores (from Zambettakis, 1970).

Table 12. First country reports of *Ustilago kamerunensis*, all on *Pennisetum purpureum*.

date	country	reference
1908	Cameroon	Ledermann, 1908
1910	Cameroon	Sydow and Sydow, 1910
1930	Uganda	Herb.* IMI 5917; Snowden, 1930
------	Congo	Watson, 1971
1963	Rwanda	Herb. IMI 102817
1975	Tanzania	Herb. IMI 192913
1991	Kenya	Herb. IMI 351419; Kung'u and Waller, in press

* Herbarium of CABI-Bioscience, formerly the UK International Mycological Institute.

It is interesting to note that McDonald, who compiled comprehensive, early host lists of Kenyan fungi, did not record *U. kamerunensis* on *P. purpureum* (McDonald, 1929, 1936).

Kung'u and Waller (in press) reported the complete destruction of the inflorescence, in agreement with the original description of Sydow and Sydow (1910). Kung'u and Waller (in press) suggested that the Kenyan strain was more virulent than those found in other East African countries, or that Kenyan varieties of *P. purpureum* were more susceptible. Apart from the original description and first reports, a literature search revealed no information on the pathogen. Nothing has been previously reported on its biology, ecology or epidemiology. There are no data concerning the effects of smut disease on *P. purpureum*, in terms of individual plant morphology or physiology, or yield loss of the crop as a whole.

2.4 The Napier grass farming system - zero grazing

Napier grass is used in zero grazing (stall-feeding or cut-and-carry) systems, where farm animals are penned at the homestead and cut grass is brought to them. Thomson (1885), during his East African exploration through the Mt Kilimanjaro region, described *"...beautiful fat animals..."* that were fed with cut forage at Taveta, and never allowed outside a fenced enclosure. Bringing in fodder from outside has been a common practice amongst the Chagga people in the same area, since prehistoric times, to reduce risks of theft and tsetse fly (Swynnerton, 1949). Other traditional reasons for penning livestock day and night were to protect against tick-borne diseases and biting flies (such as *Stomoxys* spp.), livestock control as a response to land tenure systems and land fragmentation in the absence of fencing (Boonman,1993). Traditional fodders included grasses, cereal stover, crop weeds, bean straw, tree loppings and wood ash.

In Kenya, cutting and carrying roadside grasses for penned livestock was a common sight around Nairobi in the 1960s (Boonman,1993). At that time, stall-feeding was promoted by non-governmental organisations, volunteer corps and farmer training centres, to provide employment in the densely populated peri-urban areas of the city. Stall-feeding was officially endorsed in the 1970s and donor supported programmes began to promote the practice in other parts of Kenya (Boonman,1993). This coincided with a sharp rise in milk prices after the milk quota system was abolished, which led to the adoption of Napier grass for feeding penned dairy cattle in the densely populated highlands of East and

Central Africa (where communal grazing land is restricted to the higher forest margins (Valk, 1990)). Kenyan farmers rely almost solely on Napier grass for dairy cattle, to the exclusion of other forages (M J Scarr, *pers. comm.*), though Boonman (1993) reports that other sources of fodder (such as green maize, reject grain, stover, sweet potato vines and banana residues) may comprise about 50 percent of fodder intake, when considering dairy and non-dairy cattle. Even so, any threat to productivity of an important fodder crop, such as that posed by *Ustilago kamerunensis* to Napier grass, could have a marked effect on dairy production and the incomes of small-holder farmers in Kenya.

CHAPTER 3 NAPIER GRASS PEST AND DISEASE SURVEY

3.1 Introduction

The rationale adopted for assessing smut disease of Napier grass in Kenya involved two separate surveys at different times and with different aims. Firstly, a questionnaire was distributed by mail to Kenyan government organisations in whose mandate areas Napier grass was cultivated, to assess which parts of the country were infested. Secondly, a field survey was done, in those areas that reported the disease, that assessed disease incidence and collected data on biotic and abiotic factors which may have had a bearing on the development, occurrence or severity of the disease. These data were used to model disease incidence, and also provided clues to avenues of epidemiological and ecological investigation at a later stage.

3.2 Methods

3.2.1 Postal questionnaire

For administrative purposes, Kenya is divided into provinces, districts, divisions, locations and sub-locations. A survey by Kung'u and Waller (in press) identified smut of Napier grass in several divisions of Central Province. Anecdotal reports, however, suggested that the disease was more widespread, though there was no hard evidence of its occurrence. Some growth abnormalities of Napier grass possibly caused by smut, such as early flowering and reduction in biomass, have been ascribed to pests, parasitic grasses, other pathogens or

abiotic factors (Tiley, 1969; A W Kihurani, *pers. comm.*).

A questionnaire was therefore developed to obtain information on Napier grass smut and its incidence in Kenya (Appendix 2), so that field surveys could be targeted at areas reporting the disease, and identification of the cause could be made. The questionnaire was sent to Centre Directors of the Kenya Agricultural Research Institute, and District Livestock Production Officers of the Ministry of Agriculture, Livestock, Development and Marketing, in October 1996, with a stamped addressed envelope. All Napier grass production areas were covered. Tables 13 and 14 show the responses.

Of the five positive responses, two noted that the disease was always associated with early flowering and three reported that it was spreading. Farmers thought that the method of spread was spontaneous in two cases, and through cuttings and manure in one case each. It was noteworthy that the disease was first noted in 1989 from Gatanga Division (Table 14) but was not brought to the attention of the national programme until two years later (Kung'u and Waller, in press).

Table 13. Napier grass smut disease questionnaire - responses from research centres of the Kenya Agricultural Research Institute and offices of the Ministry of Agriculture, Livestock, Development and Marketing.

centre/ office	organisation	smut reported	smut not reported	uncertain diagnosis[1]
Embu	KARI		Embu District[2]	
Embu	MoALDM		Embu District	
Kakamega	KARI			
Kakamega	MoALDM			
Katumani	KARI			Machakos District
Kiambu	MoALDM	Kiambu District[2] (Lari and Kiambaa Divisions)		
Kirinyaga	MoALDM			
Kisii	KARI			
Kisii	MoALDM			Kisii District (Mosocho Division)
Kitale	KARI			
Mtwapa	KARI			
Murang'a	MoALDM	Murang'a District[2] (Kangema Division)		
Naivasha	MoALDM			
Nakuru	MoALDM			
Nyeri	MoALDM	Nyeri District[2] (Othaya and Tetu Divisions)		
Thika	MoALDM	Murang'a District[2] (Gatanga Division)		

[1]Katumani reported absence of smut but occurrence of early flowering of unknown aetiology; Kisii reported "*infectious shrinking*" (cause unknown) and "*drying of leaves and wilting*" (ascribed to an unidentified insect pest of the root system).
[2]Names of Districts are those in place during the 1989 Kenya census.

Table 14. Napier grass smut disease questionnaire - responses from affected areas.

Division	year disease first reported	farmers' control measures	Napier grass varieties grown
Gatanga	1989	uprooting, use clean planting material	French Cameroon Bana grass, local varieties
Lari/Kiambaa	1991	uprooting, manuring	French Cameroon Bana grass
Othaya/Tetu	1993	uprooting, change to Guatemala grass, use clean planting material	French Cameroon Bana grass, local varieties
Kangema	1993	uprooting	French Cameroon Bana grass

3.2.2 Field survey

Assessing plant diseases usually involves measures of incidence (ie the percentage of infected plants in a stand and/or the percentage of infected stands in an area) and severity (where the seriousness of the disease on an infected plant is determined on the basis of the area of the affected vegetative part). Historically, for smut diseases, measuring severity is not usually done because the nature of the disease is such that the plant never produces a yield, and so only presence or absence is scored. However, in Napier grass smut, infected plants may produce an appreciable yield, in terms of biomass, depending on the extent of pathogen invasion, and so a severity score may be applicable with this particular host/pathogen interaction. For the initial field survey, incidence alone was recorded using a stratified, two-stage sampling method. The survey universe consisted of all Napier grass plots in Kenya. The survey frame was a list of sub-locations in Kiambu District, which was badly affected. The sample unit was a discrete plot of the grass, either with plants laid out along a field boundary or in a polygon.

In assessing disease incidence the proportion of affected plants is scored. To determine the sample size a proportion of 20% infested plots was assumed (based on subjective reports from other workers and personal observation).

Then, using the standard formula (as given in Poate and Daplyn, 1993):-

$$n = \frac{z^2 p(100-p)}{x^2}$$

where n = sample size, z = 1.96 (for a 95% confidence level), p = 0.2 (ie 20% incidence) and x = 0.1 (ie 10% precision), the value of n obtained was 61. Thus at least 61 Napier grass plots had to be sampled.

A list of sub-locations in Kiambu District was obtained from the most recent Kenyan census (CBS, 1993). These sub-locations were numbered sequentially. Twenty were chosen by simple random sampling (such that the total number of households to be sampled, in the sub-locations, exceeded 61) using random numbers generated by a calculator.

The survey involved a single visit to each household (ie assessment of one Napier grass plot per household), and so a varying sample size from each sub-location was acceptable (Poate and Daplyn, 1993). The number of households to be visited in each sub-location was derived using a constant sampling fraction of 0.375%, such that the number of sample households was the same proportion of the total number of households in each sub-location. The design was therefore self-weighting. Census data provided the number of households per sub-location. The plot position, in terms of longitude, latitude and altitude, was determined using the global positioning system (GPS) (using a Garmin *Survey II*), to horizontal and vertical accuracies of +/- 50m. Measurements were taken in the middle of the sample plot.

Maps of divisions were generated with mapping software (MapInfo, 1995),

using a database of the census, with land use classifications, in terms of agro-ecological zones (AEZs), superimposed on the maps to determine the AEZ of each household. AEZs were established by FAO (1978) and modified for use in Kenya by Jaetzold and Schmidt (1983). A description of the AEZ system is given in Appendix 3.

Locations of farms from the GPS were geocoded (ie *X* and *Y* coordinates were assigned to each record) and plotted on the division maps, to attempt correlation of smut presence with AEZ and soil types. The sub-locations sampled, number of households and range of AEZs, per sub-location, are given in Table 15.

Selection of households

A random letter table was used to select households, indicating R (right) and L (left) turns at road junctions. The starting point was the approximate centre of the sub-location. From the centre, the vehicle followed right or left directions according to the table, until 0.5km had been covered. The first household (right or left) with a Napier grass plot was then visited. The process was continued until the relevant number of farms had been sampled (if the border of the sub-location was reached during sampling, the vehicle was turned around and the process begun again, taking care that households were not visited more than once).

Table 15. Sub-locations sampled, numbers of households per sub-location and agro-ecological zones.

district	division	location	sub-location	no. of households to be visited	AEZ
Kiambu	Limuru	Ndeiya	Nderu	5	LH3/UM3/ LH5/LH4/ UM6
		Limuru	Bibirioni	12	LH2/UH2
	Gatundu	Chania	Gituamba	2	LH1
		Kiganjo	Kiamworia	3	UM1/LH1
		Ngenda	Gathage	4	UM2
	Kikuyu	Muguga	Kanyariri	6	LH3/UM3
		Kabete	Lower Kabete	7	UM3
	Lari	Lari	Escarpment	2	LH2
		Kijabe	Kinale	5	UH1/LH2
			Magina	3	UH2/LH2
	Githunguri	Githunguri	Kanjai	4	UM1/LH1/ UM2
			Kimathi	5	UM2
		Ikinu	Githiga	5	LH1
			Ikinu	9	UM2/UM1
			Gitiha	3	LH1
		Komothai	Kiratina	6	UM1/UM2
	Kiambaa	Kihara	Karura	13	UM3
	Thika	Juja	Kalimoni	12	UM5/UM4
		Thika	Kariminu	3	UM3
			total	109	

Napier grass data collection

The boundary length of each plot was determined using a tape measure and the area calculated. A systematic method was used to assess plant parameters and disease incidence per plot, because preliminary observation suggested clustering of diseased plants within plots. To sample a plot, a 100m tape measure was stretched across the longest diagonal as a transect line. Stations at every metre on this line were assessed. The smallest diagonal was 20m in

length and the smallest plot size was 60m^2. Diagonal sampling is appropriate for disease incidences of less than 20 percent and greater than 80 percent (Shepherd and Ferrer, 1990).

An output of the survey was the development of a model of smut disease probability. Factors that went into the model were chosen from a knowledge of the Napier grass pathosystem, and included all factors that were thought to have a biological or environmental meaning in relation to the presence of the pathogen. The factors recorded were; ground cover (many plots contained patches of bare earth between Napier grass stools), early flowering (with or without smutted heads), leaf distortion (puckering of the leaf blade and/or infolding of the distal end) and shortened internodes. Other diseases, insect pests and physiological disorders were also recorded. Some plant stems with no outward smut symptoms were cut with a knife to look for internal evidence of disease. Ten percent of plants on the transect were measured for height using a graduated pole.

Other data included the farmer's name, sub-location, age of the crop and variety, presence of smut in the field (even if not on the transect), smut history, date of sampling, source of planting material, date of last crop cut, slope of the plot (ie whether flat or sloping), identity of adjacent crops (as intercrops or in fields and boundaries, within 10m), flowering Napier grass on headlands, if appropriate, and general comments on the health of the stand.

All details were recorded on a data sheet which is shown in Appendix 4.

3.3 Field survey results

3.3.1 Analysis of survey data

Because the complete data set is complex (see Appendix 5), a summary of the major findings is detailed here. Table 16 shows the location data and numbers of responses when smut was present and Table 17 gives some of the factor results. Figure 5 shows the location of Kiambu District within Kenya. A map of the smut status of sites sampled in the District is shown in Figure 6.

Table 16. Results of survey of 109 Napier grass plots in Kiambu District, January - June 1997.

sample parameter	number	smut positive (%)
plots sampled	109	27 (25)
stations sampled	4465	
Napier grass plants sampled	3925	171 (4.4)
Napier grass plants in infested plots	1173	171 (14.6)
agro-ecological zones	9	7 (77.8)
soil types	7	2 (28.8)
sub-locations	19	10 (52.6)

Table 17. Biotic and abiotic factors from the Napier grass survey in Kiambu District, January - June 1997.

factor	range	mean	sd
age (yrs)	0.3-29	4.5	4.9
altitude (masl)[1]	1383-2650	1953	303
area (m^2)	20-3158	530	679
density (plants/hectare)	6691-18500	16530	2258
height (m)	0.2-3	0.9	0.6
when cut (months before survey)	-9 to -1	-2.9	1.7
other pests and diseases	aphids, leaf hoppers, leaf spots (all minor)		
adjacent crops	maize, beans, horticultural and cash crops		
source of Napier grass	neighbours or within farm (98%)		
variety	not known (100%)		
normal flowers	none		

[1]masl - meters above sea level

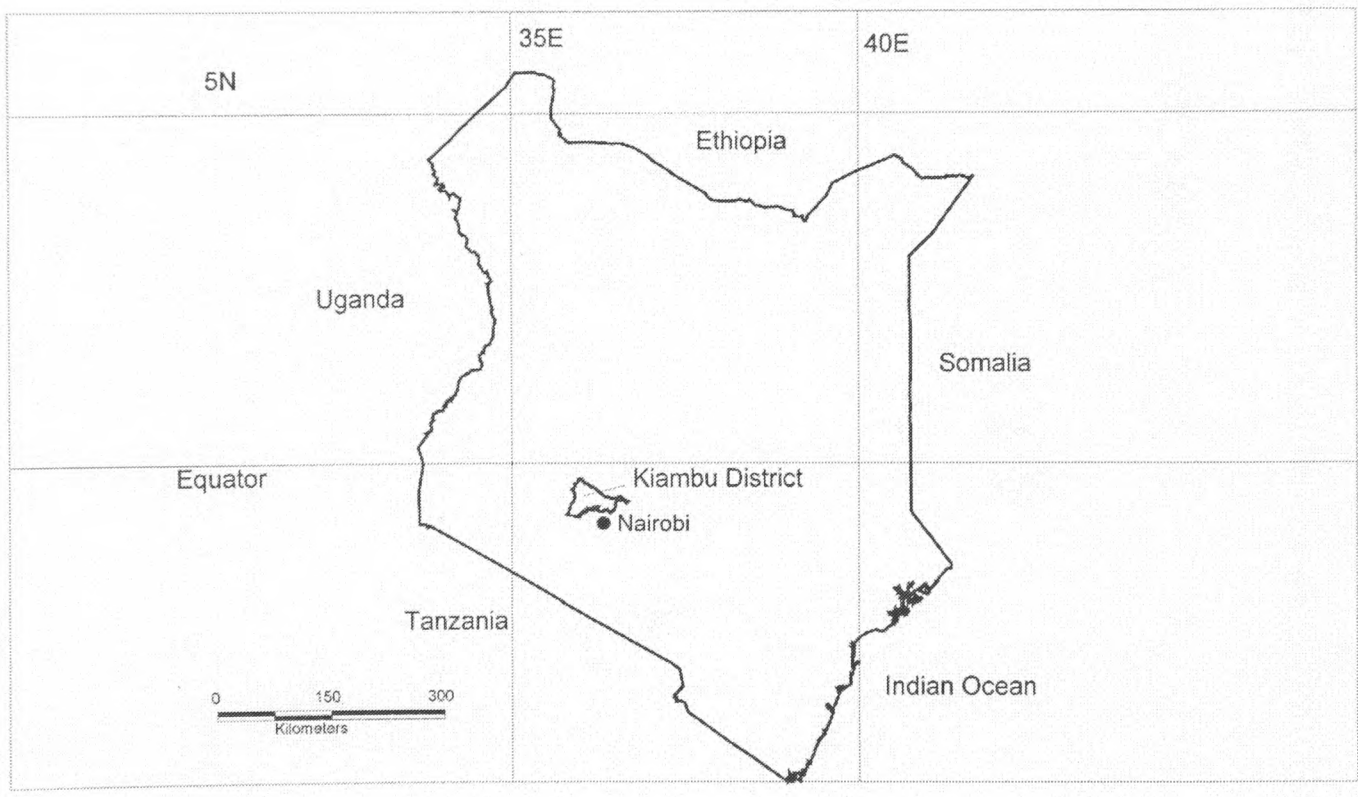


Figure 5. Map of Kenya showing the location of Kiambu District.

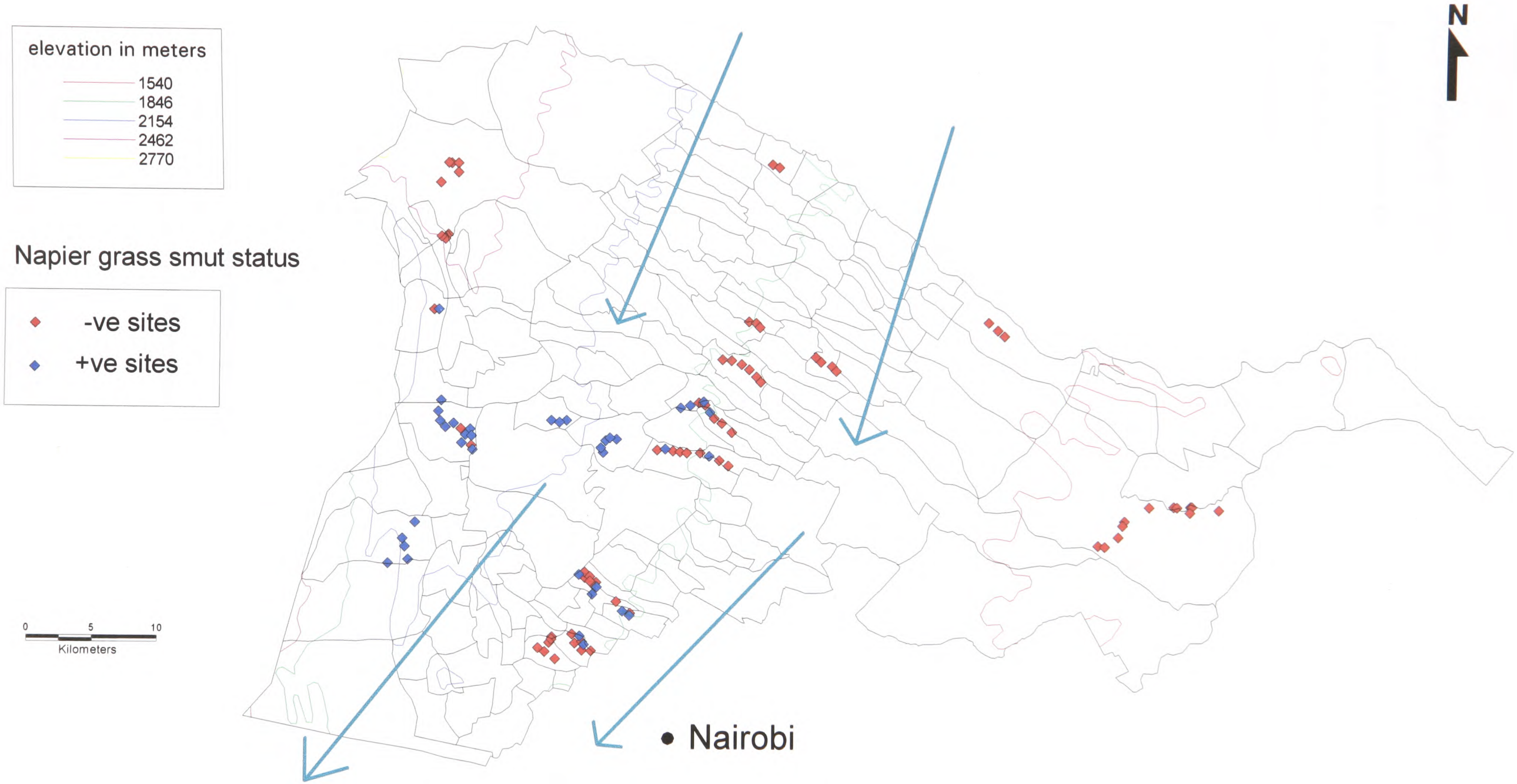


Figure 6. Map of Kiambu District showing sub-locations, Napier grass smut status and prevailing wind direction.

Agro-ecological zones

Table 18 shows the AEZ characteristics of the zones that were sampled. The disease was found in most AEZs and sub-locations. Agro-ecologies that had the infection were in the upper midland, lower highland and upper highland zones. Only upper highland 1 and upper midland 5 did not have the disease.

Plant density

Of interest from Table 17 was the fact that many plots had gaps in the rows where plants had died and not been replaced. The plant density recommended by MoALDM is 18,500 plants per hectare. Plots with lower densities were not realising their maximum potential for Napier grass production. Some farmers practised infilling, and so the age of the stand (ie from when it was first established) does not necessarily reflect the age of all the plants within the stand.

Other pests and diseases

No other pests or diseases of major importance were found. Insects included nymphal stages of leafhoppers (Cicadellidae), aphids (*Macrosiphum euphorbiae* Thoms and *Sitobion* Mordvilko spp.), ants (*Pheidole megacephala* F.) and mites (*Tetranychus* spp.). The mites were associated with bronzing of the underside of the leaf blades. Fungal foliar pathogens were *Helminthosporium* spp., *Pyricularia* spp. and *Beniowskia sphaeroidea*. Table 19 shows the percentage incidence of insects and diseases.

Table 18. Agro-ecological zones and belts of zones sampled, with agronomic factors.

zone belt	zone	no.[1]	farm enterprise	temperatures	mean annual rainfall (mm)	altitude (masl)	comments
UH	1	5	sheep/dairy	annual mean 10-15°C	2200-2500	2130-2430	seasonal night frosts
UH	2	14	pyrethrum/wheat	annual mean 13-15°C	950-1200	2130-2380	
LH	1	10	tea/dairy	annual mean 15-18°C mean minimum 8-11°C	1300-2000	1820-2280	normally no frost
LH	2	2	wheat/maize/ pyrethrum	annual mean 15-17°C	900-1300	1980-2280	
LH	3	10	wheat/barley	annual mean 16-17°C	800-1200	1950-2070	
UM	1	9	coffee/tea	annual mean 18-21°C mean minimum 11-14°C	1300-1600	1700-1820	
UM	2	19	coffee	annual mean 18-20°C	1000-1400	1580-1760	
UM	3	27	marginal coffee	annual mean 19-20°C	800-1200	1520-1580	
UM	5	12	livestock/sorghum	annual mean 20-21°C	600-730	1360-1520	

[1] number of records

Insect feeding damage consisted of notching of leaf margins, as caused by caterpillars or grasshoppers, and windowing of leaf blades, which is typical of larval stem borer activity. No examples of these insects were seen. The puckering of unfolding leaf blades was of unknown aetiology. Some smutted plants showed this type of leaf distortion but it was not correlated with smut presence.

Table 19. Incidence of insects and fungal diseases on 3925 Napier grass plants.

organism	% incidence
ants	0.9
aphids	0.1
mites	7.8
leafhoppers	4.0
termites	0.1
insect damage*	5.3
Beniowskia	2.1
Pyricularia	0.2
Helminthosporium	18.3
leaf puckering	3.8

* physical damage to leaves caused by insect feeding

Soils

The FAO-Unesco soil grouping system for classifying soils was used (FAO-Unesco (1974) as given by Ellis and Mellor, 1995), with amendments applicable to Kenya (FURP, 1987). Table 20 shows the soil classifications and descriptions of the households that were sampled. Smut was found in Napier grass plants on soils LP1, RB2 and RB3.

Table 20. Soil classes and descriptions of sampled households.

soil[1]	no.[2]	description[1]	soil class[3]	connotation[3]
RB1	2	well drained, extremely deep, dark reddish brown to dark brown, friable with humic andosol and slightly smeary clay, with acid humic topsoil	ando-humic nitisol,	shiny ped faces, dark surface horizon (rich in volcanic glass)
RB2	64	well drained, extremely deep, dusky red to dark reddish brown, friable clay, with acid humic topsoil	humic nitisol	shiny ped faces
RB3	23	as for RB2 but with inclusions of well drained, moderately deep, dark red to dark reddish brown, friable clay over rock, pisoferric or petroferric material	eutric nitisol, with nito-chromic cambisol and chromic acrisol and luvisol, partly lithic	shiny ped faces, changes in colour, low base saturation, clay accumulation
LP1	3	well drained, moderately deep to very deep, dark brown, friable and slightly smeary, clay loam to clay, with humic topsoil	ando-luvic phaeozem	organic rich, dark colour
UP1	4	well drained, very deep, dark reddish to dark brown, very friable and smeary silty clay loam, with humic topsoil	mollic andosol	dark surface horizon
LB8	5	imperfectly drained, very deep, dark grey to black, firm to very firm, bouldery and stony, cracking clay, in places with calcareous, slightly saline deeper subsoil	pellic vertisol	surface soil turnover
VC	7	complex of; well drained to poorly drained, shallow to deep, dark reddish brown to black, firm, silty clay to clay, in places calcareous and/or cracking, rocky and stony	gleysol fluvisol cambisol vertisol etc	excess water alluvial deposit colour, structure surface soil turnover

[1] FURP,1987 [2] number of records [3] Ellis and Mellor,1995

In the FURP classification, the first letter refers to the physiography and the second letter to the lithology, hence R - volcanic footridge; B - basic and ultra-basic igneous rock; U - uplands, upper, middle and lower levels; P - pyroclastic rock; L - plateau and high level structural plain; V - minor valley; C - complex

Adjacent crops

Adjacent crops included all those commonly found in high potential areas, though maize, cabbage and banana predominated (see Table 21).

Source of Napier grass planting material and types grown

Almost all farmers obtained Napier grass from within the farm or from neighbours, and none knew which type they were cultivating. This lack of awareness is a reflection of the ubiquity and non-commercialisation of the grass. Although its value is recognised, few farmers undertook any husbandry to improve yields. It is only with the arrival of the smut disease that farmers began looking for advice on Napier grass cultivation and management.

Napier grass height and date last cut

The low crop heights were attributed to the poor rainy season. Plant growth was poor with low biomass and there was a heavy demand for fodder as a result, which was leading to frequent cutting (with cutting intervals of two weeks or less, in some cases).

Slope of plot

Sixty-six plots were flat. No attempt was made to measure the angle of sloping plots but it varied from approximately 5° to 45°.

Table 21. Crops and habitats adjacent to Napier grass in sampled plots.

crop	number of records	comment
banana	38	
cabbage	26	
coffee	15	
fallow	11	
flowers	2	
French beans	19	
fruit and trees	22	loquat, passion fruit, mango, pawpaw, citrus, avocado, pears, grevillea
horticulture	5	spinach, sweet potato, kale, rhubarb
intercrops	5	maize, French beans or cabbage
maize	59	
Napier grass	18	
peas	8	
potato	16	
roadside	9	

Single instances of castor oil (*Ricinus* L. sp.), pyrethrum and sugarcane were also recorded.

Spatial pattern analysis of smut incidence within plots

The discrete character of smut diseases enables spatial pattern analysis to be done, to determine whether there is a random or non-random occurrence of the disease within a plot (ie are diseased plants clustered, indicating that plants adjacent to those diseased are more likely to be infected than those further away). Ordinary-runs analysis (Madden *et al.*, 1982) was used to detect clustering. A run is defined as a succession of one or more diseased or healthy plants. The observed number of runs will be less than the expected number of runs if clustering has occurred. The Z test (where $n > 20$) is used to determine clustering, with a value of Z less than -1.64 indicating clustering, at $p = 0.05$.

The analysis was carried out on data collected along the transects, for plots with at least three smutted plants. The transect point was every metre ie about 110% of the inter-plant distance along the rows. The results are given in Table 22.

Table 22. Ordinary runs analysis of Napier grass plots with smut disease.

plot no.	no. infected plants per transect	total no. plants per transect	observed no. of runs	expected no. of runs	sd	*Z* statistic[1]
11	10	60	13	17.7	2.104	-1.98*
13	29	66	13	33.5	3.971	-5.04*
17	7	37	9	9.6	1.356	-0.11
18	3	41	3	6.6	0.796	-3.84*
26	11	40	14	17.0	2.473	-0.99
35	36	92	34	44.8	4.542	-2.27*
38	3	38	7	6.5	0.822	1.18
39	3	27	7	6.3	0.943	1.24
40	4	29	5	7.9	1.205	-1.99*
43	7	49	11	13.0	1.658	-0.90
48	3	25	5	6.3	0.971	-0.80
60	3	43	5	6.6	0.780	-1.39
61	11	38	15	16.6	2.486	-0.46
70	4	46	8	8.3	1.012	0.19
71	11	56	15	18.7	2.315	-1.37
74	7	36	9	12.3	1.820	-1.53
75	6	26	7	10.2	1.743	-1.57
84	3	44	7	6.6	0.776	1.18

[1] A continuity correction of 0.5 was added to the numerator of *Z* (Gibbons, 1976).
* significant at $p = 0.05$

3.3.2 Modelling the incidence of smut disease of Napier grass

Disease incidence is recorded for smuts, such that responses to surveys are coded *yes* (for smut presence) or *no* (for smut absence). In such cases, when attempting to establish relationships between disease incidence and biotic and abiotic factors, a binomial distribution of incidence is obtained, and so a

generalised linear model (GLM) is appropriate for data analysis, with smut presence as the response variable (other methods of grouping data, such as factor analysis, were not applicable since they treat no single variable as a response (Payne *et al.,* 1987)). The distribution of the factors is not relevant to GLM, because the aim is to estimate the probability of a site having the disease. A wide spread of data points is therefore advantageous.

The procedure adopted was to start with a maximal model in which all the individual factors were included. Interactions of factors that might have had a biological meaning were also calculated, but none of these interaction terms was significant, and so they are not presented. The model was derived from an analysis of deviance (using the Genstat 5 statistics package (Payne *et al.,* 1987)). Observation of the results of the fitted model showed which factors were contributing significantly (the significance of the deviance ratio was assessed from its χ^2 value at $p = 0.05$, using standard statistical tables). Non-significant factors were then removed from the model one at a time, and the analysis run again after each removal to observe the effect of dropping the factor. This process continued until only significant factors remained in the model. The final model, therefore, was the simplest one that explained the probability of disease incidence.

Table 23 shows the results of the analysis of deviance with all factors included. Plant density, plant height, adjacent crops, slope of the plot, AEZ, soil type,

plot area and when the plants were last cut were all found to be non-significant. These factors were therefore dropped from the regression analysis. At this stage the age of the stand was no longer significant and so only altitude remained as a significant factor (Table 24). The best fit of the regression line was achieved by including altitude as a quadratic function. The equation of the model is shown in Figure 7 and the estimate of the correlation coefficients in Table 25. The fitted line is given in Figure 8.

Table 23. Accumulated analysis of deviance, showing the effect of all factors on the proportion of Napier grass smut.

change	df	deviance	mean deviance	*p*
+ AEZ	8	28.9983	3.6248	ns
+ age of stand	1	9.1175	9.1175	<0.01
+ altitude of stand	2	11.6732	5.8366	<0.1
+ area of stand	1	0.0114	0.0114	ns
+ cereal/Napier grass [1]	1	3.4261	3.4261	<0.1
+ cash crop[1]	1	0.321	0.321	ns
+ roadside[1]	1	0.005	0.005	ns
+ fruit trees[1]	1	0.6539	0.6539	ns
+ horticultural crops[1]	1	0.716	0.716	ns
+ Napier grass density	1	0.4281	0.4281	ns
+ Napier grass height	1	0.6483	0.6483	ns
+ slope of stand	1	2.1453	2.1453	ns
+ soil[2]	6	5.7756	0.9626	ns
+ when last cut	1	0.1408	0.1408	ns
residual	66	60.055	0.9099	
total	93	124.1156	1.3346	

[1] adjacent crops or roadside
[2] soil type is included for completeness although the standard errors were very large. The Genstat algorithm was unable to deal with the soil data set in which most observations were of one soil type.

Given that the observed residual deviance (60.1) is very similar to its degrees of freedom (66), and that the residual mean deviance (0.91) is approximately

equal to unity, it is unlikely that any important factors have not been considered in the model (Cox and Snell, 1989).

Table 24. Accumulated analysis of deviance, showing the significant factors of the proportion of Napier grass smut.

change	df	deviance	mean deviance	*p*
+ altitude	2	39.8995	19.9497	<0.01
residual	105	97.5876	0.9294	
total	107	137.4871	1.2849	

Table 25. Estimate of the regression coefficients for the model of proportion of smut disease of Napier grass.

	estimate	se	$t_{(\infty)}$	*p*
constant (*a*)	-100.6	24.8	-4.05	<0.001
altitude linear (*b*)	0.093	0.0235	3.96	<0.001
altitude quadratic (*c*)	-0.00002123	0.0000055	-3.86	<0.001

$$\log\left(\frac{p}{1-p}\right) = a + (b)altitude + (c)altitude^2$$

Figure 7. Equation of Napier grass smut probability.

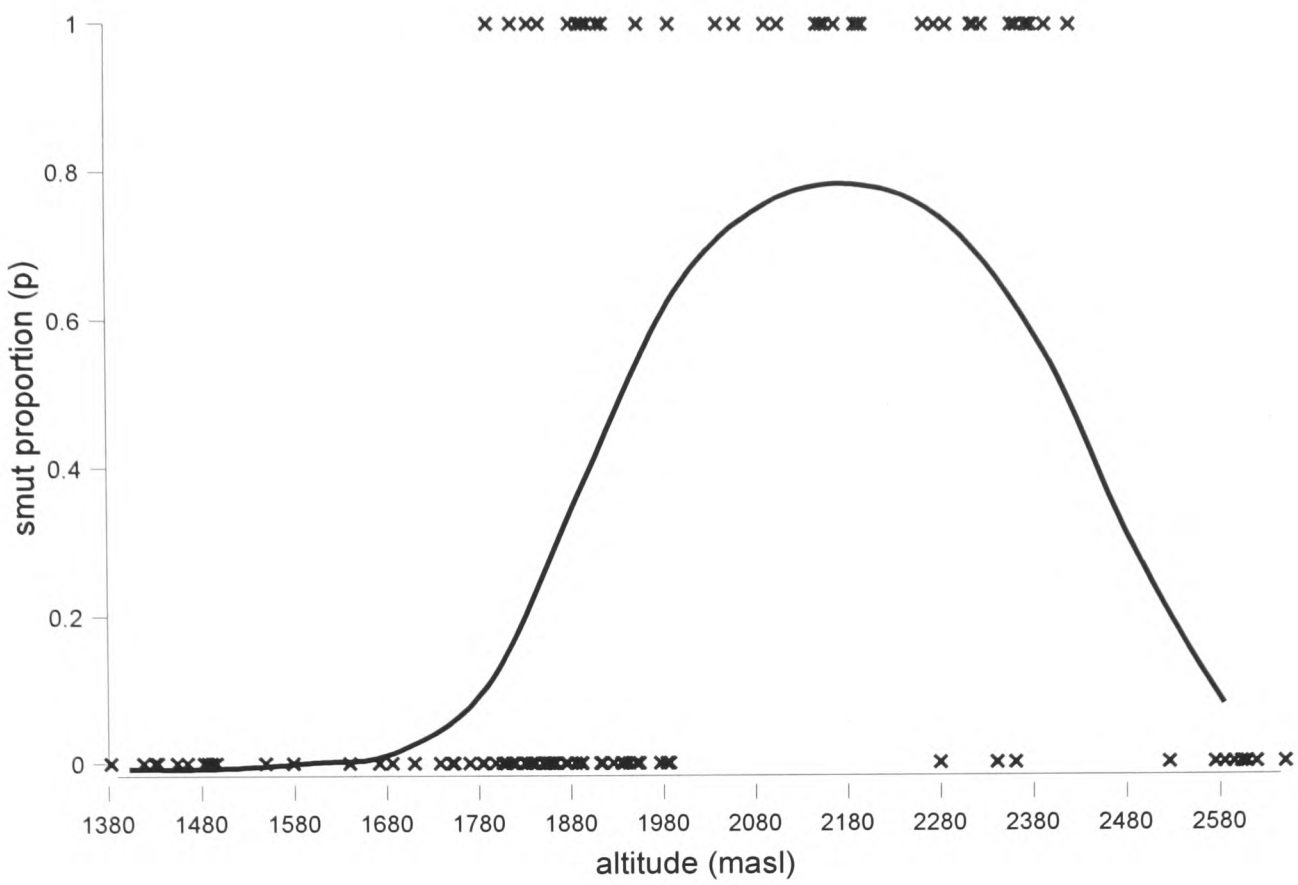


Figure 8. Fitted and observed relationship of altitude and the proportion of Napier grass smut disease (every x represents a sampled plot. If smut proportion = 0 then no disease was seen in the plot; if smut proportion = 1, then the disease was present).

3.4 Discussion

The disease survey was limited to Kiambu District (this being the most heavily infected district, and the most convenient to sample, considering the poor state of the roads during the survey period). However, further work confirmed that the disease is confined to the Central Highlands, in the Lower Highland, Upper Midland and Upper Highland agro-ecological zone belts (Scarr, 1998). Kiambu District, in which these zone belts are found, was thus representative of the agro-ecologies from which the disease has been reported countrywide, and

which provide the most favourable bio-climatic conditions for the growth of Napier grass (Jaetzold and Schmidt, 1983).

Although smut disease of Napier grass was reported from Uganda and Tanzania more than twenty years ago, it seems to have taken many years for the disease to become noticeable in Kenya. A diseased inflorescence will produce many millions of spores, which are very light and can be spread long distances by the wind. Windborne spores are the major means of dispersal in the smut fungi, sometimes over large distances (Simmonds, 1994). However, it is intriguing to note that the first reported outbreaks in Kenya were not found adjacent to the Napier grass growing borders of Uganda and Tanzania, as might be expected if wind dispersal was the major factor in disease spread. The predominant wind direction in Kiambu District is from the north-east, as shown in Figure 6.

Also of interest is the apparent clustering of the outbreak in Kiambu District, with a similar cluster in Nyeri (G Karanja, *pers. comm.*). This suggests that the source of the Kenya outbreak may have been infected planting material. The survey revealed that most farmers obtained Napier grass from neighbours or within the farm. Since many farmers (27 per cent) were not aware that the disease was present on their plots, its spread via infected splits or canes could have gone unnoticed.

Spatial pattern analysis revealed non-clustering of diseased plants in 72% of the plots, suggesting that plant-to-plant transmission of the disease is of minor importance, and that diseased plants do not act as major foci of infection within a plot. The lack of clustering also suggests that it is not necessary to carry out systematic sampling within the plot.

Models for disease forecasting, based on biotic and environmental factors, have been developed for many plant pathosystems. Campbell and Madden (1990) list 32 crops for which models have been proposed; that of Waggoner (1968) for late blight on potato being the earliest. However, none of these forecasting models are for smut diseases. Some work has been done on smut of sugarcane, including that of Elston and Simmonds (1988), who constructed a model of probability of infection and Momol *et al.* (1990), who produced disease gradients. Amorim *et al.* (1993) and Hau *et al.* (1993) attempted to differentiate sugarcane cultivars, with artificial and natural infection, using monomolecular and Gompertz functions. Theories on the population dynamics of *Ustilago violacea* (Persoon) Roussel on *Silene alba* L., where insects are the vectors of the fungus, have been put forward by Thrall and Jarosz (1994), Roche *et al.* (1995), Thrall *et al.* (1995) and Alexander *et al.* (1996). However, all of these smut models are ecologically based and none deal with environmental factors. An extensive literature search suggests that the model proposed here for Napier grass smut is novel, in that it offers an environmental explanation as a basis for forecasting the probability of a smut disease. Thus

it is appropriate to recommend that further surveys undertaken by other investigators should use the survey methodology reported here. Results will then be comparable with existing work, though it should be noted that some factors identified here as not significant may not need to be recorded, while other factors, such as cutting frequency and levels of manuring, could be added to the model.

Furthermore, underlying parameters that could contribute to the effect of altitude need elucidation. They may comprise temperature, rainfall, some other unknown factor or a combination of these. Rainfall patterns are likely to be particularly important, since they alter the presence of the free water needed for bud growth and spore germination, and have been found to influence the development of other smut diseases. For example, cool, humid or wet conditions promoted infection of wheat by the Karnal bunt fungus, *Tilletia indica* (APS, 1998), and spores of the flag smut pathogen (*Urocystis agropyri*) germinated optimally in soils at moisture contents that favoured wheat seed germination (Line, 1998).

High rainfall (at higher altitudes) may reduce soil fertility (and possibly increase disease susceptibility) through leaching of soil nutrients. It is possible that fertilizer application could compensate for leaching, although when Lusweti (1998) assessed fertilizer application and cutting frequency on disease incidence the results were inconclusive, since the effects varied depending on

the site of the trial plot. Any beneficial effects of fertilisation could be confounded by plant stress arising from farm management activities, such as weeding and cutting, and cutting induces tiller bud formation that provides opportunities for the pathogen to infect the host. Further analysis of the data may be possible, but ideally a larger data set is required. For example, in the present AEZ list, there are only two examples from Lower Highland zone 2, one of which had the disease whereas the other did not, and this precludes any meaningful interpretation.

The model proposed here suggests that the disease may be contained at altitudes of 1800-2500m, though this needs to be confirmed by further surveys in other areas at different altitudes. The grass is cultivated from sea level up to 2800m, though the altitude range recommended for its production in Kenya is 800m-2000m (Jaetzold and Schmidt,1983). Altitudes above 2000m suffer from occasional night frosts that badly damage the grass. Napier grass will grow at the coast but loses vigour after two or three years (D M Miano, *pers. comm.*), presumably because of the high daytime temperatures in excess of 30°C. Information on locations with high disease probabilities, on the basis of altitude, would be useful in targeting recommendations for the management of the disease to those areas at particular risk.

The derived model was intended to describe an observed relationship between smut incidence and various factors, ie it is an empirical or correlative type. It

was considered more appropriate than the explanatory type because the construction of a comprehensive explanatory model was thought infeasible in the absence of information on all possible contributing factors. In addition, an empirical model derived from survey data does not require advance knowledge of dependent and independent variables (Campbell and Madden, 1990).

The model can also be classified as of the predictive type, that is used to forecast the likelihood of a particular area being at risk from smut disease of Napier grass. It may be that the final model derived from the variables is composed of several sub-models. For example, the variable altitude could be considered a function of rainfall, temperature, spore germination, soil antagonists or other factors. Elucidation of sub-models would allow construction of a systems, or simulation, model.

Finally, although the development of disease management strategies is at an early stage, anecdotal evidence suggests that cultural control is already being practised by some farmers, involving roguing of diseased plants and manuring to improve plant vigour. Investigations to identify tolerant or resistant Napier grass types, and assess the susceptibility of other fodder species such as Guatemala grass (*Tripsacum fasciculatum* Trin ex Asch., synonyms *T. laxum* and *T. andersonii*) are reported below.

(The work described in this chapter was presented as a poster at the 7th International Congress of Plant Pathology, in Edinburgh, from 9th to 16th August 1998. The abstract is given as Appendix 6.)

CHAPTER 4 THE PATHOSYSTEM

A collection of known *P. purpureum* types in Kenya was established at the KARI Muguga National Research Centre by colleagues working on their use as fodders. This museum was the source of healthy experimental material for the trials reported below.

4.1 External morphology of smut-infected Napier grass

In healthy Napier grass plants the stems may be 8m tall, with leaves up to 150cm long and 4cm wide. The inflorescence can be 30cm in length and plants have a well developed but shallow root system. Tillering and stolon production may be profuse, depending on the type. Growth after cutting is rapid and vigorous (Häfliger and Scholz, 1980). In contrast, infected stems are much thinner and shorter than normal, showing induced dwarfing (being less than 1.5m in height), with leaves reduced in number and size. Severely infected stems have shortened internodes, increased aerial tillering and a fasciculate appearance. The leaves may be distorted and regrowth after cutting is slow. Table 26 shows the characteristics of some smutted stems, collected at random during the field survey of Napier grass plots (Chapter 3).

Table 26. Characteristics of smutted (n = 768) and healthy (n = 106) stems of Napier grass*.

	mean length (cm)	range (cm)	sd
stem, not including inflorescence[1]	39.80	1.5-128	19.04
length of diseased inflorescence[2]	9.88	3.0-18	2.78
length of smutted portion of inflorescence[3]	9.33	0-17.8	2.89
length of healthy inflorescence	18.94	10.2-30.8	5.71

* healthy inflorescences were less common in farmers' plots because the stools were not usually allowed to reach their maximum height (4-5m), at which the inflorescences appear.
[1] from ground level to the proximal sorus or spikelet on the inflorescence. In diseased stems, approximately 94% of the inflorescence was smutted.
[2] from the proximal sorus or spikelet to the tip of the inflorescence.
[3] from the proximal to distal sorus on the inflorescence.

Severely affected stools have a chlorotic and stunted habit. Tillering at soil level is limited or non-existent, and badly infected stools subsequently wither and die. The root system is also reduced and plants can be easily pulled out of the ground.

Depending on the type of Napier grass, flowering varies from uncommon to very common in healthy plants (see Table 2), but is usually only found on mature individuals (Boonman, 1993). However, premature flowering may be induced by *U. kamerunensis*, with the emerging inflorescence being more or

less smutted. Napier grass plants with precocious inflorescences were locally described as exhibiting *early flowering.*

The infected inflorescence is smaller than normal and emerges smutted from the sheathing leaves, with all or part of it converted to a mass of black smut sori and spores (an example is shown in Figure 9). A naked rachis is left after spore dispersal. On an infected stool, the number of smutted stems ranges from one to many. Comparison of means from Table 26 revealed that healthy inflorescences were significantly longer than smutted ones (t = 21.71, $p < 0.0001$).

Figure 9. Photograph of an inflorescence of Napier grass infected by *Ustilago kamerunensis.*

There are no other physical signs of damage to the host and no other parts of the plant show any macroscopic fungal structures. Figure 10 shows the gross morphology of infected plants, and Figure 11 shows the fasciculate appearance of a severely diseased stem.

Figure 10. Comparison of smutted Napier grass plants. All stems are infected in the small plant on the left. The plant on the right is 2m tall and has only two infected stems.

Figure 11. Severely infected stem of Napier grass, showing the short internodes and fasciculate appearance. The stem is 1m tall.

4.2 Internal morphology of smut-infected Napier grass

Stem sections were cut and stained to confirm the presence of fungal structures within the host.

4.2.1 Methods

The method was taken from Lloyd and Pillay (1980), which was developed to assess sugarcane smut resistance. Stems from Napier grass plants of unknown type were cut in 2cm long cylinders between the nodes. Each cylinder of tissue was halved and then quartered lengthwise. One 0.5cm x 2cm x 1mm section (including the epidermis) was cut from each of the two tissue faces at right angles to each other on the quartered stem section. Sections were cleared by boiling in lactophenol for ten minutes, and then stained in 1.5% cotton blue in lactophenol for five minutes. Stained sections were mounted on microscope slides.

4.2.2 Results

Under microscopic examination (x400), blue-stained intercellular hyphae and haustoria were revealed. Colonizing hyphae were found in the xylemparenchymatous tissues. Haustorial mother cells developed as thickenings of normal hyphae. The mother cells produced a short lateral branch, at right angles and of similar diameter (the haustorial neck). The haustorial neck gave rise to haustoria within the cells. Haustoria were densely aggregated. Figure 12 shows the stained fungal structures within Napier grass tissues.

Stained preparations were also made from stems with fully formed but precocious smut-free inflorescences, to determine whether infection could be asymptomatic. However, no internal infection was found in these sections.

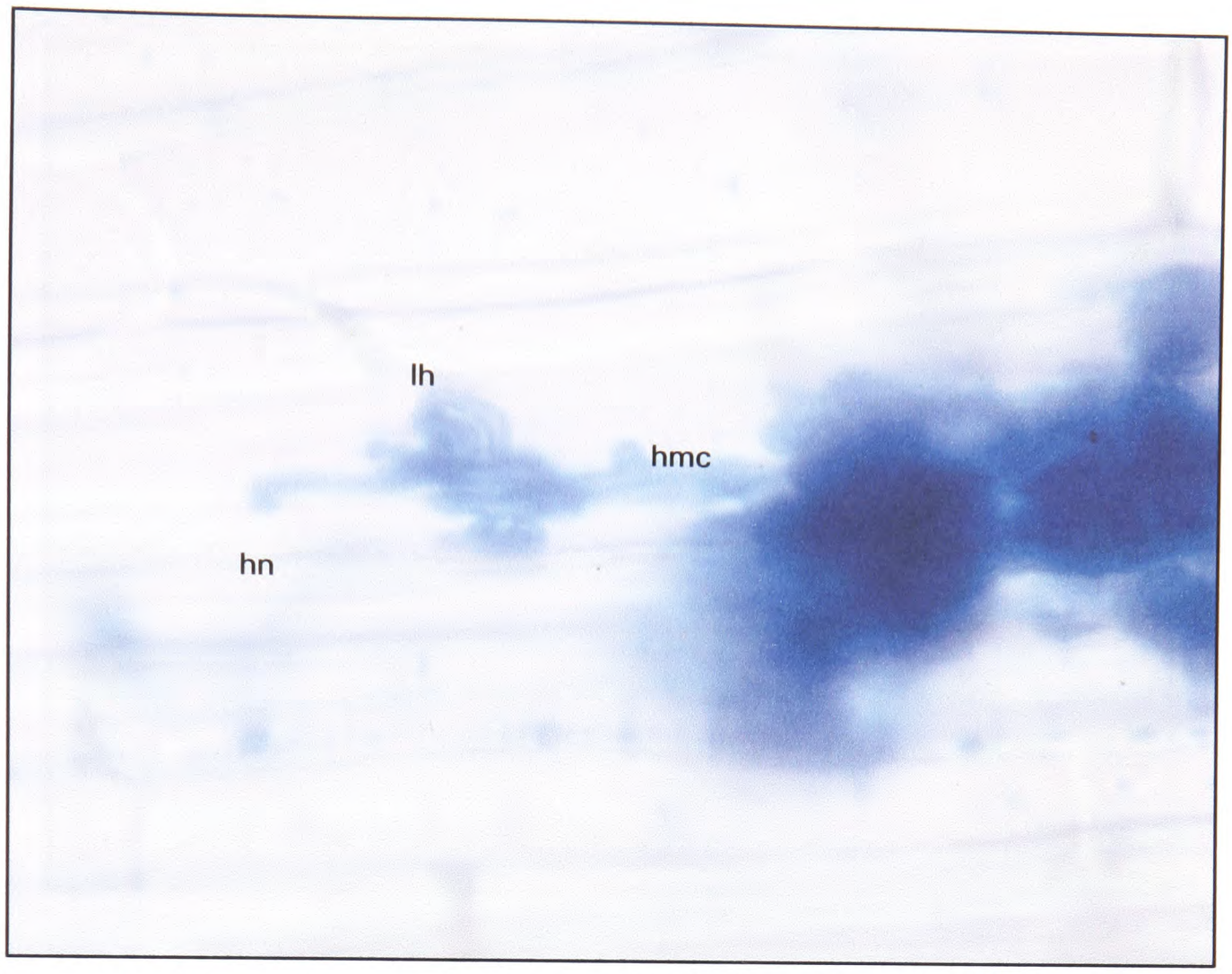


Figure 12. Photomicrograph of a longitudinal section of a Napier grass stem; hn - haustoria neck, hmc - haustorial mother cell, lh - lobed haustoria (stained with 1.5% cotton blue in lactophenol, x1250 magnification).

4.3 Ustilospores of *Ustilago kamerunensis*

Ustilospore suspensions were made by shaking a smutted inflorescence in distilled water. Drops of the suspension were placed on slides, under coverslips, and examined microscopically. The ustilospores were smooth, globose, slightly flattened, thin walled and light brown in colour. The mean maximum diameter of 50 spores, measured using a calibrated eyepiece graticule, was 7μm (range 5.7μm – 8.7μm, sd 0.52μm). The mean minimum diameter was 6.7μm (range 5.2μm – 7.8μm, sd 0.54μm). Figure 13 shows the germination products from ustilospores of *U. kamerunensis*.

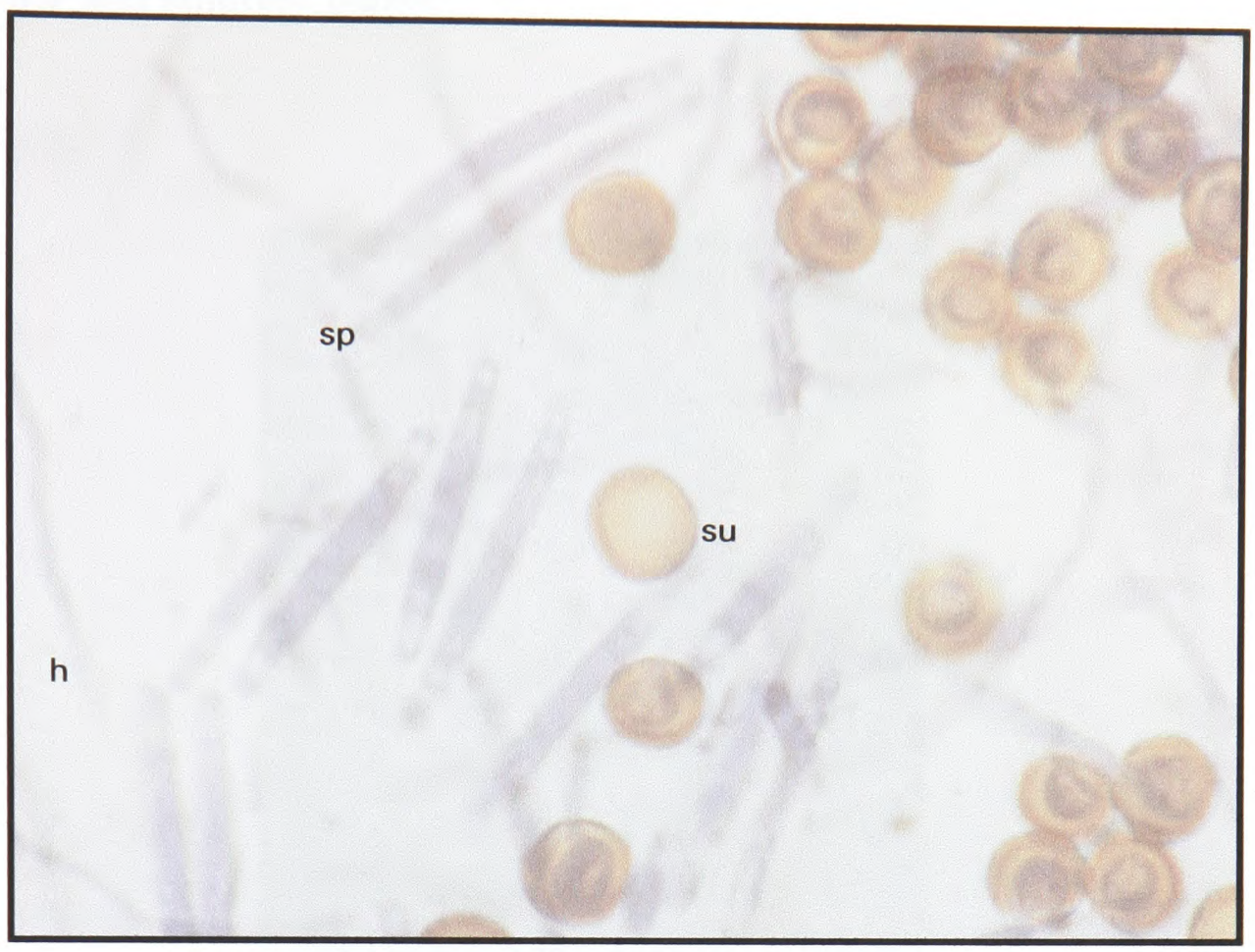


Figure 13. Photomicrograph of *Ustilago kamerunensis* germination products; sp - sporidia, su - sterile ustilospore, h - hypha (stained with 0.5% aqueous trypan blue, magnification x1250).

4.4 The sorus of *Ustilago kamerunensis*

In infected plants, the ovaries, stamens, glumes and lemmas were transformed into spore-bearing sori, leaving only a few hairs on the spikelet unaffected. Microscopic examination of sori revealed no peridia, collumellae or sterile filaments. The spores were loosely aggregated and not catenulate. Fifty sori from different inflorescences were measured. The mean length was 4.5mm (range 2.5mm - 9.0mm, sd 1.37mm) and mean width was 0.98mm (range

0.5mm -1.5mm, sd 0.26mm). Figures 14 and 15 show inflorescences from healthy and smutted plants.

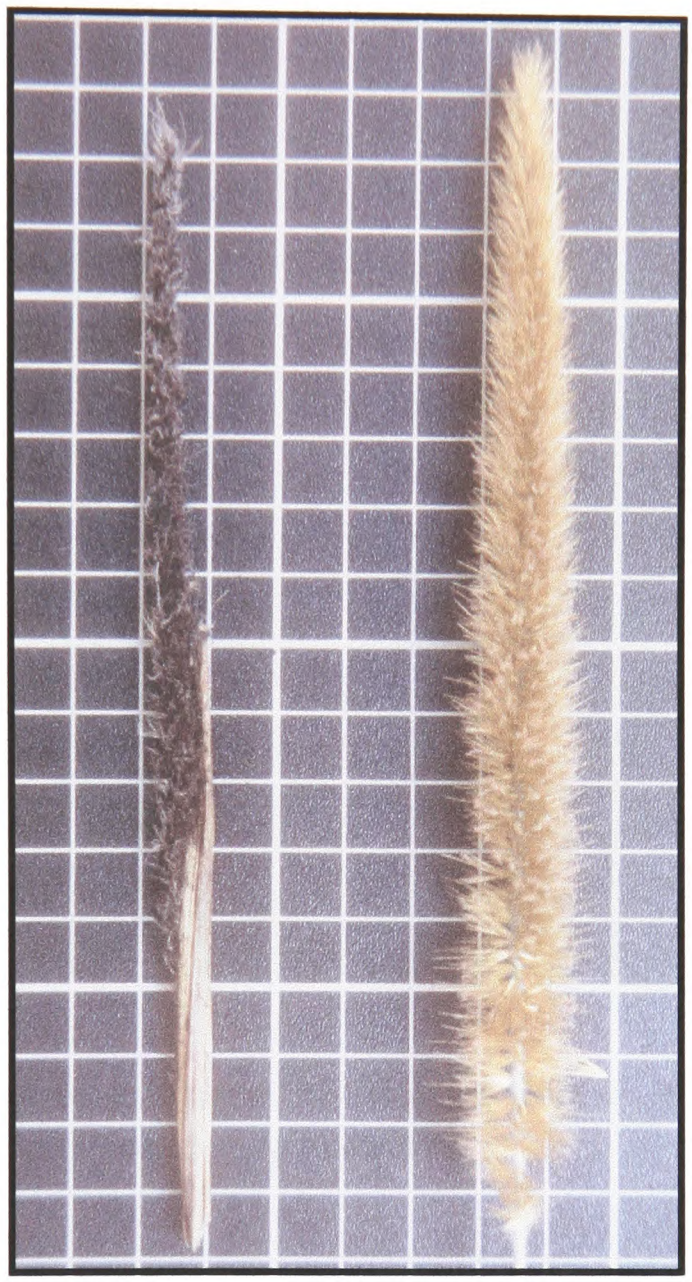


Figure 14. Healthy (right) and smut infected (left) inflorescences of *Pennisetum purpureum* (the background grid is 1cm squares).

Figure 15. Magnified image of healthy (right) and smut infected (left) inflorescences of *Pennisetum purpureum*.

Fischer and Holton (1957) reported several cases in which the number of sori was greater than the number of spikelets that would have been produced if the plant had not been infected. Comparison was therefore made between the numbers of sori of *U. kamerunensis* and the number of healthy spikelets on uninfected inflorescences of *P. purpureum*. Using a dissecting microscope, the number of sori and spikelets per cm were counted on 50 inflorescences selected at random from three *P. purpureum* types that were in flower in the

Muguga collection. It was assumed that one spikelet was transformed into one sorus. The healthy samples were pooled, so that comparison could be made between healthy and diseased spikelets. The results are shown in Table 27. Analysis of variance revealed significant differences between the number of diseased and healthy spikelets per cm of rachis ($F_{(1,144)} = 114.5, p < 0.0001$).

Table 27. Mean numbers of spikelets per cm on healthy and smut-infected types of *Pennisetum purpureum.*

Napier grass type	mean no. of spikelets	range	sem
healthy	24	18-35	0.35
diseased	18	12-26	0.48

4.5 Cultural characteristics of *Ustilago kamerunensis*

4.5.1 Ustilospore germination

Ustilago kamerunensis is not an obligate parasite and so it was possible to grow colonies on agar media in Petri dishes. Smutted inflorescences were held over exposed agar surfaces and gently tapped to dislodge ustilospores. Tap water agar, Oxoid malt extract agar (0.2% and 2%) and Oxoid potato dextrose agar (PDA) were used. The plates were incubated at room temperature and colonies developed within three days, although the rate of growth was slowest on tap water agar, it being the least nutritious medium. On all media the colonies reached a maximum diameter of 1cm. Colonies were white and floccose. The reverse was pale cream.

Spore germination was studied microscopically using the method devised by Ingold (1983). After dusting the surface of the agars, 1cm cubes of agar were cut from the dish using a sterilized needle. The excised cubes were put on microscope slides, with the spore-dusted surface uppermost, and a flame-sterilized coverslip placed on top. Germination could then be examined directly. Ustilospores germinated within eight hours with variable germination products, but usually involving a promycelium as the first stage. On weakly nutritious media, such as tap water and 0.2% malt extract agars, the promycelium developed into a long, branched or unbranched, septate germ tube, with only the distal cell containing cytoplasm, and the rest being hyaline. These hyphae did not produce sporidia. Occasionally a short promycelium developed from an ustilospore, which gave rise to several sporidia. On other occasions a single sporidium was produced directly from the ustilospore, with no promycelium being visible. After detachment of the first sporidium, a second sporidium was sometimes produced. If such sporidia developed from a promycelium, then this development must have occurred inside the ustilospore. At a later stage some sporidia budded laterally, in the manner of yeast cells, but only on more nutritious media such as 2% malt extract and PDA. Most sporidia contained vacuoles. No anastomoses or clamp connections between any of the germination products were observed.

Fifty-eight sporidia were measured. Their mean length was 15.5μm (range 7μm - 26μm, sd 2.9μm) and mean width was 1.68μm (range 0.9μm - 2.0μm,

sd 0.13μm).

Stained preparations of the germination products were made. Flame sterilized cover slips were pressed lightly on spore-dusted agar surfaces and then transferred to drops of stain on microscope slides. General structures were examined using aqueous 0.5% trypan blue and 1.5% cotton blue in lactophenol. Preparations to examine nuclei were made using Giemsa stain (Johnston and Booth, 1983), which revealed sporidia containing one or more nuclei (sporidia sometimes had one or more septa). Nuclei were assumed to be haploid. Figure 13 is a photomicrograph of the ustilospore germination products and Figure 16 shows a series of computer generated drawings of spore germination over time.

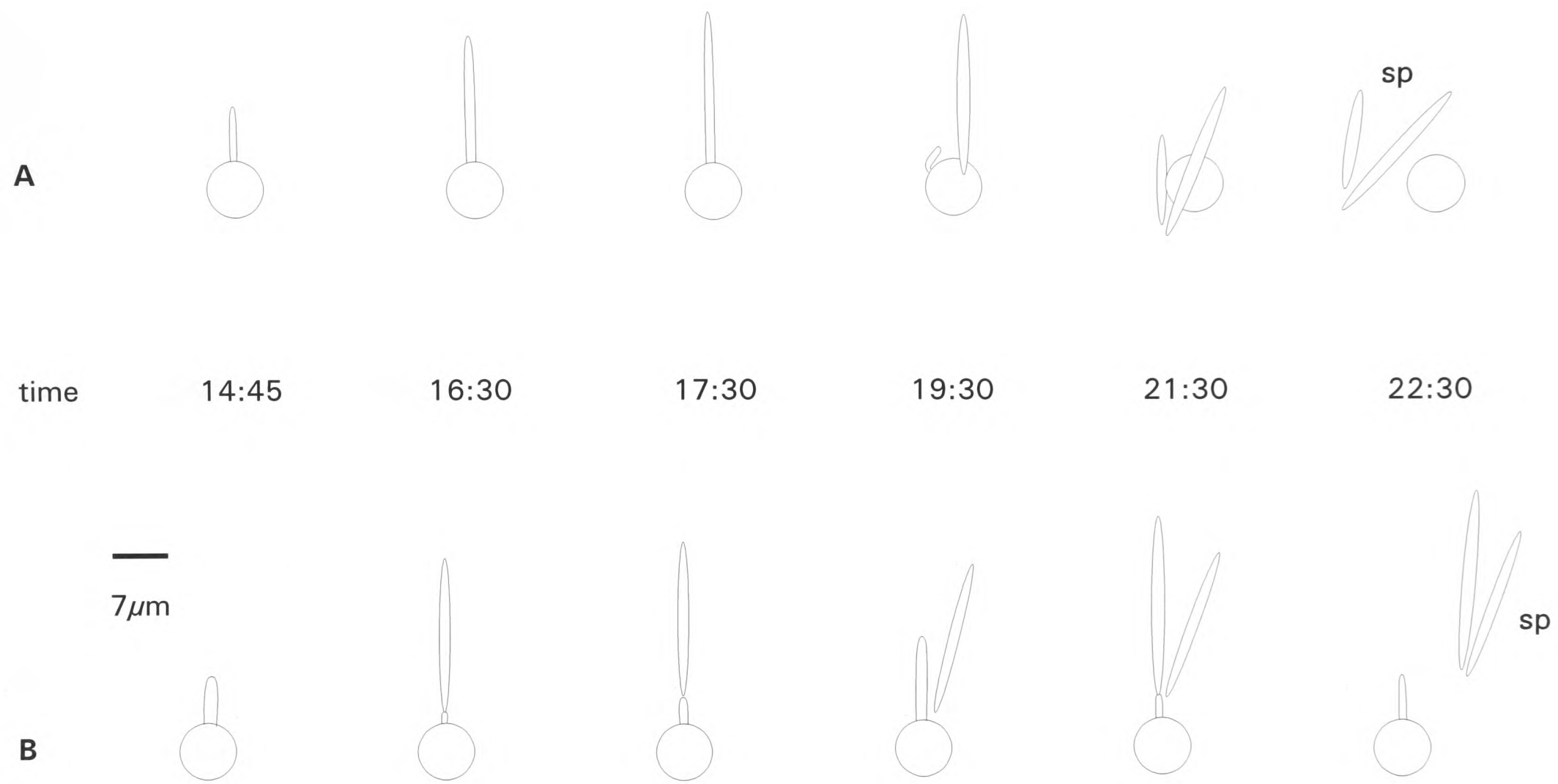
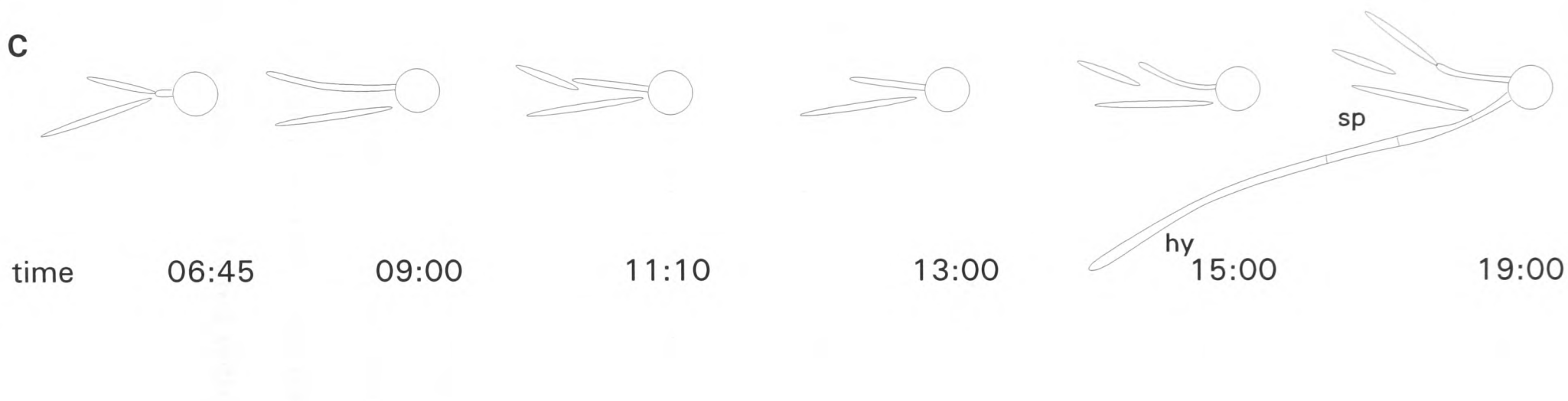


Figure 16. Computer drawn images of the germination products of two spores (A and B) of *Ustilago kamerunensis*. Spores were deposited on 0.2% malt extract at 08:00 (sp - sporidia).



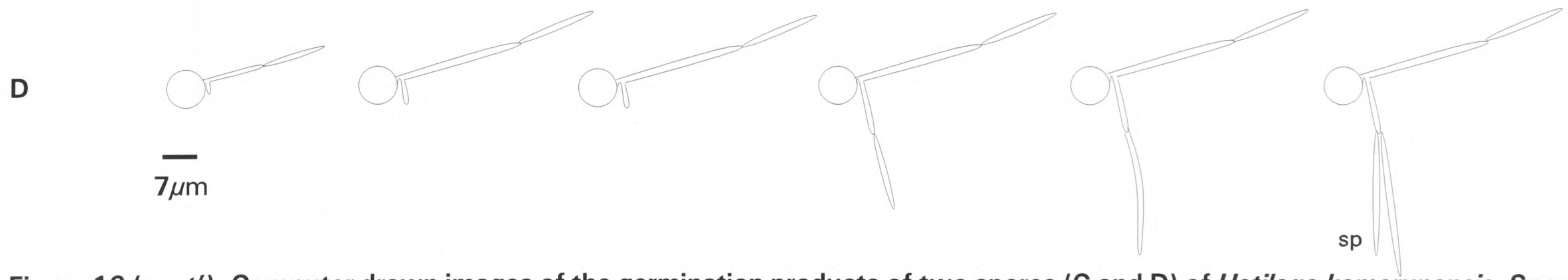


Figure 16 (cont'). Computer drawn images of the germination products of two spores (C and D) of *Ustilago kamerunensis*. Spores deposited on 0.2% malt extract agar at 22:00 the previous day (sp - sporidia, hy - hypha).

4.6 Discussion

The major morphological changes induced by smut infections were given previously in Table 7 (page 23). In the genus *Ustilago*, induced hermaphroditism, heterostyly, dehiscence, dwarfing, phyllody and tillering, and reduction in the root system have been reported (Fischer and Holton, 1957). Of these, induced tillering and dwarfing, and reduced roots were found in Napier grass plants infected by *Ustilago kamerunensis*. Fasciculation, shortened internodes and leaf distortion were also sometimes observed. Morphological modifications were restricted to stems that gave rise to smutted inflorescences. It was possible, therefore, if only one or a few stems were infected, for the root system of the whole stool to look relatively undamaged, and for adjacent uninfected stems on the same stool to maintain a healthy appearance.

It was not possible to determine any effects on the flower parts of Napier grass because almost all of the spikelet was converted to sori and spores. Results suggested that smutted plants produced inflorescences with fewer spikelets per cm, though the smutted inflorescences were collected from a population of mixed Napier grass types. This may have masked the contribution of a type with less than the average number of spikelets (a search of the literature revealed no information on numbers of spikelets in *Pennisetum* species). Increased flower production has also been described in *Viscaria vulgaris* L. (=*Lychnis viscaria* L.), infected with the anther smut *U. violacea* (Jennersten, 1988).

It was noteworthy that some farmers did not recognise the changed inflorescence morphology of smutted plants as being caused by a disease. They simply assumed that it was a new type of the grass. Similar confusion has occurred in the past. Fischer and Holton (1957) reported several instances of smut induced inflorescence modifications that misled botanists to conclude they had come across new species or varieties. Smut-induced dwarfing led Linnaeus to describe common *Agrostis tenuis* as a new species, *A. polymorpha* var. *pumila*, and the species *Stipa hassei* was abandoned when it was later discovered to be a smutted plant of *S. eminens* var. *andersonii* Vasey (Fischer and Holton, 1957).

However, early flowering was the first symptom of smut infected Napier grass reported by farmers. The precocious inflorescence was usually smutted, but occasionally, healthy inflorescences arose from healthy stools or stools on which other stems were diseased. In uninfected plants, flowers are normally formed on mature stools approaching their maximum height. As reported above, stems supporting healthy inflorescences did not show internal fungal structures, suggesting that early flowering may have been induced by physiological stress, such as drought or increased cutting frequency, or as a response to smut infection in other parts of the stool.

It may be the case that early flowering is not in fact occurring. Severely infected stools, with flowers, are stunted but may be the same age as non-

infected adjacent plants which are of normal size. The presumption is made by farmers that the dwarfed plant is younger and, therefore, that any flowers it produces are precocious. However, the phenomenon of early flowering induced by plant disease has been described in other hosts. For example, Singh and Misra (1974) used gibberellic acid to prevent early flower induction in chrysanthemum, caused by chrysanthemum stunt virus. Precocious flowering in some *Vinca* L. sp. resulted from infections by *Puccinia vincae* Berk., and in *Cirsium arvense* (L.) Scop. attacked by *P. suaveolens* (Persoon) Rostrup (Horsfall and Dimond, 1959). Another instance was reported by Jennersten (1988), when *Viscaria vulgaris*, infected by *U. violacea*, bloomed earlier and remained open longer than healthy plants. This smut is distributed by pollen gathering bumblebees of the genus *Bombus* Latreille, and Jennersten (1988) speculated that early flowering forced inexperienced bees to visit infected flowers, thus becoming disease vectors even though they prefer healthy flowers later in the flowering season.

As with most other smut fungi (Fischer and Holton, 1957), the mycelium of *U. kamerunensis* was systemic and intercellular. The hyphae were septate and branched, and produced lobed and curved haustoria as feeding structures inside host cells.

There was no evidence of any defensive structures produced by the host, such as thickened or invaginated cell walls. Lloyd and Pillay (1980) did find such

defensive mechanisms in some resistant varieties of sugarcane. They also described different haustorial forms in resistant and susceptible tissues. In resistant varieties, haustoria were tightly coiled with many short lobes, whereas colonized tissues had fewer, long and loosely coiled lobes in xylemparenchyma and single, very long spiralled lobes in tracheids. The presence of defensive structures in Napier grass would have provided evidence of the existence of post-infectional resistance or tolerance to *U. kamerunensis*, but none were observed in this Napier grass type.

Ustilospores and sori of *U. kamerunensis* were typical of those described for the genus *Ustilago* in appearance and size. The diameter of ustilospores in this genus was given by Mordue and Ainsworth (1984) as between 4μm and 18μm and by Vánky (1987) as from 5μm to 9μm, occasionally up to 10μm. The ustilospores of *U. kamerunensis* were sub-globose and about 7μm in diameter. This dimension is within the limits of 6μm to 8μm, as reported in the first description of *U. kamerunensis* spores by Sydow and Sydow (1910). The sori were confined to the spikelets and the ustilospores formed a blackish-brown, adhering mass. Ustilospores were loose and powdery at maturity and the spore mass was easily disrupted by light pressure. No fungal structures other than the ustilospores were observed and there were no signs of spines on young ustilospores, which have been reported in *U. tricophora* (Link) Kunze, prior to pigmentation of the walls (Fullerton and Langdon, 1969). Ustilospores were uniform in colour, with no equatorial bands or polar caps as have been

described in some species of *Ustilago* (Duran, 1973). Peridia were not found, though they are present in *U. hordei* and *U. nuda* (Langdon and Fullerton, 1975). Langdon and Fullerton (1975) proposed that the presence of host tissues around the sorus was related to the stage of development of the host organs at the time of fungal invasion, the speed of hyphal spread (which could be constrained by internal mechanisms of resistance) and the susceptibility of flower tissues to destruction. The fact that partial inflorescence destruction sometimes occurs with *U. kamerunensis* may also be explained by these factors, or it may be related to the presence or absence of hyphae in the apical meristem at the time of flower initiation.

According to Vánky (1987), germination of ustilospores gives rise to a four-celled promycelium, in which karyogamy occurs and which produces sporidia on agar media. In a series of papers, Ingold (1983, 1984, 1985, 1986, 1987, 1988,1989), described the germination products of several *Ustilago* species. As part of this series, Ingold (1989) produced a comparative review of spore germination, in which a basic germination pattern was described. This consisted of the production of a straight germ tube from the ustilospore which becomes divided by three visible septa, giving rise to a four celled metabasidium or promycelium. Sporidia then bud from each of the cells. This process is modified in some species so that the proximal cell of the promycelium may remain in the spore, one septum may be produced instead of three, or the promycelium may fragment or branch and some of its cells may

fail to produce sporidia. Of particular relevance here was *U. kuehneana* Wolff, in which the promycelium was very long, with basal vacuolation which drove the protoplasm into the distal end of the germ tube. In *U. kamerunensis*, all of these variants were seen. Ingold (1989) suggested a developmental pattern based on the germination products, with the basic pattern of a four celled promycelium exhibited by *U. bistortarum* (De Candolle) Schröter. Using Ingold's criteria, *U. kamerunensis* is most closely allied to *U. aschersoniana* Fischer de Waldeim, in that both sometimes produce long germ tubes on tap water agar, though evidence of conjugation is lacking in *U. kamerunensis*. Ingold (1989) described different germination products when different media were used. This was also the case with *U. kamerunensis*, in which different germination products were produced on nutritionally weak and rich media. There was no evidence that growth on artificial media would progress beyond the formation of sporidia or hyphae. It appears, therefore, that the life cycle of *U. kamerunensis* cannot be completed outside the host.

CHAPTER 5 EPIDEMIOLOGY OF *USTILAGO KAMERUNENSIS*

5.1 The infection court

5.1.1 Introduction

The first demonstration that smut spores were infective, and hence the causal agents of plant diseases, was made by Tillet in 1755. Even so, it took over a century before the infective nature of smut spores was accepted. Early workers established four modes of infection, namely seedling infection (as in bunt of wheat), embryo infection (such as *U. violacea* invasion of *Viscaria vulgaris*), shoot infection (for example *U. scitaminea* on sugarcane and *U. kamerunensis* attacking Napier grass) and local infection (as found in corn smut) (Fischer and Holton, 1957). The first three are examples of systemic infections and the latter describes an infection localised within the host tissues.

Much work has been done on shoot infection in sugarcane smut, particularly by Bock (1964) and Waller (1967, 1969, 1970) in East Africa. They tried various inoculation methods to induce disease and elucidated the site of entry of the pathogen into the host, the infection court. They also established the importance of bud morphology in the resistance of sugarcane to *U. scitaminea*, and suggested various techniques for testing disease resistance to facilitate the selection of immune varieties.

Because resistant types of Napier grass are thought to offer the most cost

efficient means of disease management for small-holder farmers, similar investigations were done on *U. kamerunensis*, to determine whether the same type of resistance mechanisms operated in Napier grass exposed to attack by this pathogen.

5.1.2 Methods

The method described was modified from that of Waller (1970). Clone 13 was used for the trial, since this Napier grass type has been shown to be particularly susceptible to the disease (see Chapter 7). Stems were taken from plants of a known type from the Muguga collection and cut into three-node lengths. The short stem pieces were laid in trays of moist sand so that they were half immersed, and examined daily for bud germination (this is the standard technique for germinating sugarcane buds (J M Waller, *pers. comm.*)). Once bud elongation became apparent, the outer bud sheathing leaves were removed to expose the buds on ten stems. A drop of suspension, containing about 500 ustilospores, was then placed on various surfaces of the bud and stem. The stems were afterwards incubated for 24h, at 30°C and 100% relative humidity. Figure 17 shows the sites of spore inoculation.

After incubation, tissue sections were cut from bud and node surfaces to which the spore suspension had been applied. The sections were stained in Bell's reagent (0.1% trypan blue in 80% chloral hydrate (Bell, 1951)) and examined microscopically for germination products.

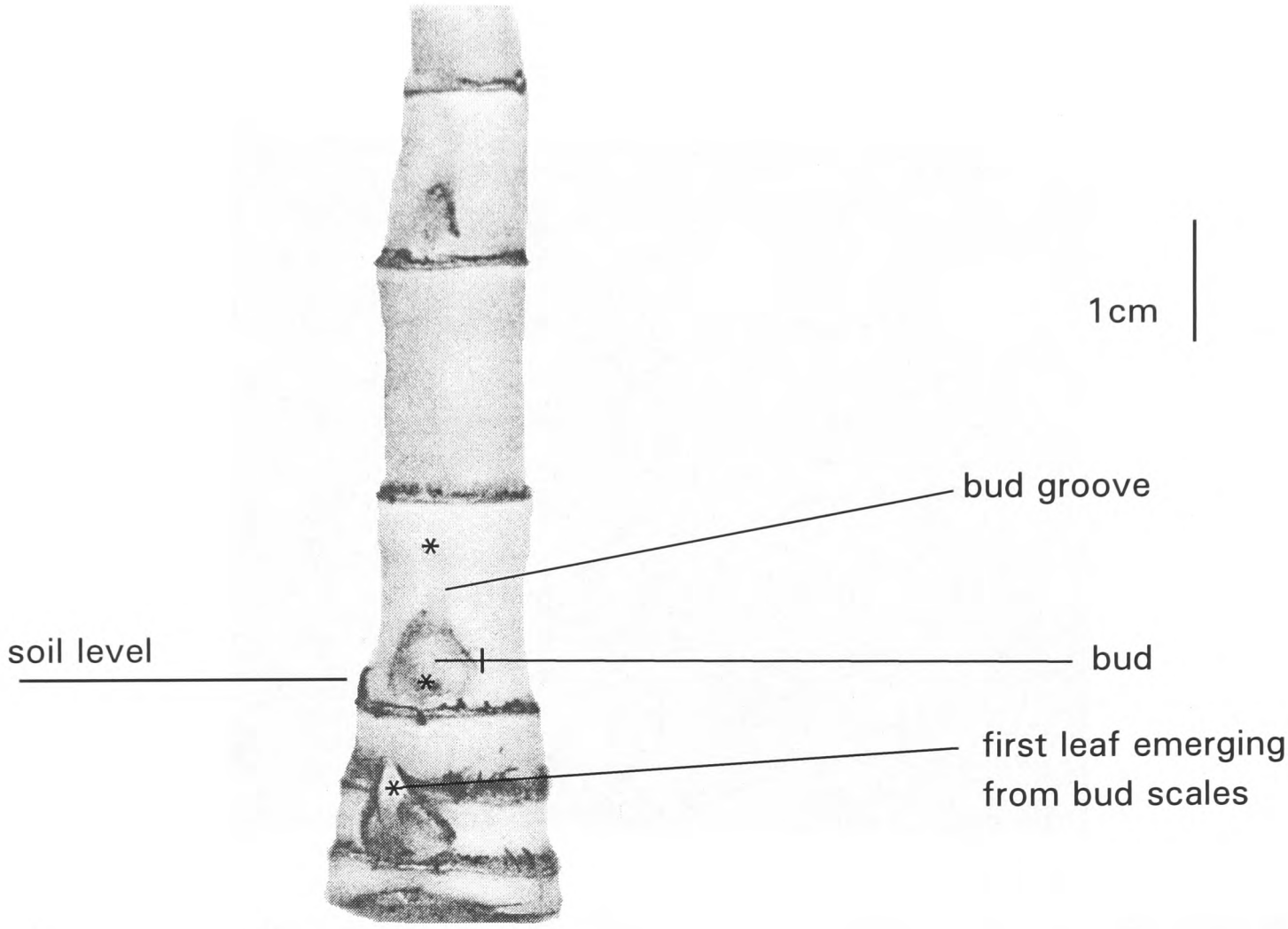


Figure 17. A scanned image of a Napier grass stem, with sheathing leaves removed, showing the sites (*) of ustilospore application.

5.1.3 Results

Extensive examination of the bud and nodal surfaces revealed plentiful production of sporidia and hyphae (about 50μm in length), from ustilospores at all inoculation sites. Sporidia were also produced on stem surfaces that were damaged. However, appressoria were restricted to the meristems of the buds,

just behind the tip. They were not found on nodal surfaces nor at the bases of germinating buds. The appressoria were swollen, ovoid to circular and borne at the ends of short germ tubes 20μm long, at an angle to the germ tube. They were darkly stained, and an example is shown in Figure 18.

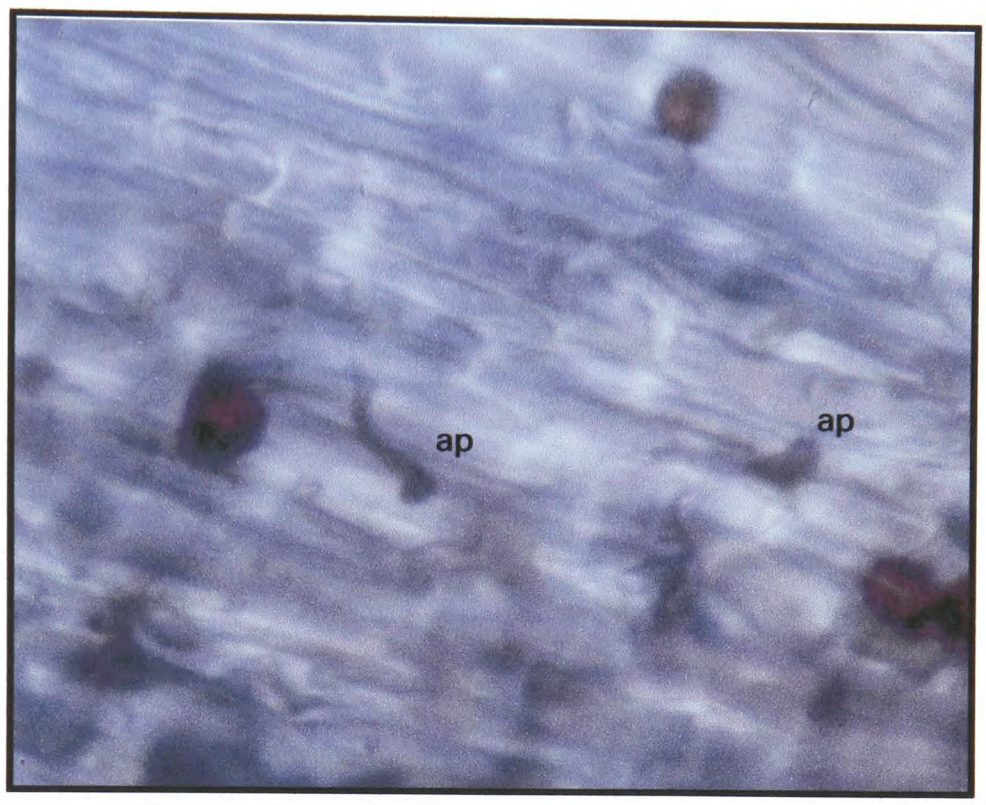


Figure 18. Appressoria (ap) of *Ustilago kamerunensis* on the surface of meristematic bud tissue of Napier grass (stained with Bell's reagent, x 425 magnification).

From ten replications, the mean number of ustilospores that produced appressoria was 10.8%. Neither infection pegs nor invasion hyphae could be seen, presumably because the appressoria were not mature enough to have produced such structures, as evidenced by the presence of stained cytoplasm within the appressoria. J M Waller (*pers. comm.*) reported that infection hyphae of *U. scitaminea* could be seen beneath 36h old, more or less empty appressoria.

5.1.4 Discussion

Appressoria were not produced when ustilospores germinated on agar but only on host tissues with an undifferentiated epidermis. Possibly there is some stimulus arising from the meristematic tissue that induces the formation of appressoria. It is not understood what function, if any, the hyphae had in the infection process, since they appeared to be distinct morphologically, at least in length, from the germ tubes that produced appressoria. Similarly, the function of the sporidia was not determined. Waller (1970) discovered that sporidia of *U. scitaminea*, placed on meristems, initiated infection from appressoria that were formed after anastomosis of sporidial germ tubes. It is possible that similar processes exist in *U. kamerunensis.*

There was no evidence of appressoria forming over stomata, which would provide an easier method of ingress than having to force an infection peg through the plant cuticle and epidermis. It appears that ustilospores germinating on plant surfaces have sufficient stored energy to produce hyphae, sporidia or appressoria at the ends of short germ tubes, but not to maintain a prolonged existence on the outside of the host, nor to be able to obtain any nutrients when prevented from gaining access to internal tissues using haustoria. Feeding haustoria were only formed from mycelia inside the host.

It has been postulated that appressorial formation could be used to assess resistance of different Napier grass types, although Waller (1970) reported that

there were no differences in percentage appressorium development in several sugarcane varieties of differing susceptibility. This suggests that physical barriers to infection by *U. scitaminea* and *U. kamerunensis* are provided by the tight sheathing leaves that surround the dormant buds, and/or by the buds' undifferentiated state or because of hardening of the tissues. This protection is removed when the soft, differentiating bud emerges from the sheath leaves and comes into contact with ustilospores. In Napier grass, buds remain quiescent until dormancy is broken when the plants are harvested. When buds push through soil carrying *U. kamerunensis* ustilospores they come into contact with spores that are then able to cause infection. It seems, therefore, that there is a narrow window of opportunity for infection by the pathogen, and that this explains the ability of mature, uncut Napier grass, to remain free of infection.

There is some discussion as to the whether the term appressorium is appropriate for the smut fungi. According to Emmett and Parbery (1975), the appressorium is the "...*expression of the genotype during the final phase of germination... as long as the structure adheres to and penetrates the host.*" In Ainsworth and Bisby's *Dictionary of the Fungi* (Hawksworth *et al.,* 1995), the appressorium is ".. .*a swelling on a germ tube or hypha, esp. for attachment in an early stage of infection...*". Common to both definitions is the belief that the appresorium is the direct precursor to invasion of the host, mediated through the production of an infection peg, and it is in this context that Waller

(1970) used the term when describing infection of sugarcane by *U. scitaminea*. However, J E M Mordue (*pers. comm.*) suggests that smuts do not use appressoria to infect their hosts, relying instead on infection from basidia or from within glume sheaths or as mycelia within the seed. Mordue describes smut fungi as being very economical in their life cycles, as nearly every limited scrap of mycelia they produce is transformed into spores. Nonetheless, if *U. kamerunensis* is only able to infect Napier grass through undifferentiated tissues, and the infection process appears, morphologically at least, to conform to the accepted description of appressorium, it seems acceptable to retain the use of the term for *U. kamerunensis*.

5.2 Spore survival

5.2.1 Introduction

As was shown in the previous section, the infection court for *Ustilago kamerunensis* is the tiller bud of Napier grass, with germinating buds being most susceptible to infection from ustilospores. One factor which can affect the development of a smut epidemic is the concentration of viable ustilospores in the soil. The number of viable spores is affected by proximity to the source of inoculum and edaphic conditions (such as temperature and moisture) that influence spore germination (and hence spore viability or longevity). This was demonstrated by Hoy *et al.* (1991) with sugarcane smut disease. Influential conditions may include light, temperature, moisture, soil pH, age of the spores, nutrient availability and fungistatic effects.

In particular, the longevity of ustilospores of *U. kamerunensis* could well have a bearing on the management of the disease. For instance, if the spores survived for short periods then it could be appropriate to recommend a fallow period so that contaminated land freed itself of infestation. Ustilospore longevity is also an important factor in the epidemiology of smut disease outbreaks.

It was suggested in Chapter 3 that temperature could be one of the underlying parameters that contributed to the effect of altitude in the model of smut proportion. Fischer and Holton (1957) stated that temperature is an important factor to understand as one of the influences of smut spore germination, and so an experiment was set up to assess the viability of ustilospores stored at different temperatures, and to determine their ability to germinate at these temperatures. In addition, it was necessary to assess, under natural conditions, the ratio of spores which germinated periodically. To provide comparison with reports on smut spore viability from other workers, that are based mainly on herbarium material, the survival rate of *U. kamerunensis* ustilospores as air dried material was also investigated. It can be seen in Table 28 that large variations in spore longevity have been reported, from less than one year for *U. tritici* and *U. nuda*, to more than 60 years for *U. crameri*.

Table 28. Longevity of smut spores in different environments.

species	longevity period (years)	environment	reference
Ustilago tritici	<1	herbarium	Holton *et al.*, 1968
U. nuda (Jens.)	<1	herbarium	Holton *et al.*, 1968
U. scitaminea	<1	soil	Andreis, 1980; Mansour *et al.*, 1991; Hoy *et al.*, 1993; Suzuki *et al.*, 1994
U. nuda	1	freeze dried	Kratka and Ujevic, 1974
U. avenae	1-13*	herbarium	Fischer, 1936
U. bullata	2-10*	herbarium	Fischer, 1936
U. hordei	2-23*	herbarium	Fischer, 1936
Urocystis colchici (Schlect.) Rabenh.	<3	ambient	Tachibana and Duran, 1966
Cintractia junci (Schwein.) Trelease	3	herbarium	Fischer, 1936
Sorosporium melandryi Sydow	3	herbarium	Fischer, 1936
U. nuda f. sp. *tritici*	3	liquid air (-189°C)	Joshi *et al.*, 1974
Urocystis agropyri (Preuss) Schröter	3/4	soil/seed	Mordue and Waller, 1981
Ustilago syntherismae (=*U. rabenhorstiana*)	3-7*	herbarium	Fischer, 1936
U. neglecta Niessl	3-7*	herbarium	Fischer, 1936
U. spegazzinii var. *agrostis* Fisch. & Hirschh.	3-12*	herbarium	Fischer, 1936
C. caricis (Pers.) Magnus	4	herbarium	Fischer, 1936
U. kolleri (=*U. levis*)	4	herbarium	Fischer, 1936
U. scitaminea	4	refrigerated	Leu, 1972
Tilletia indica	4/5	soil/seed	Goates, 1998
Tilletia foetida (=*T. levis*)	4-25*	herbarium	Fischer, 1936
U. cynodontis	5	herbarium	Fischer, 1936
U. mulfordiana Ellis & Everhart	9	herbarium	Fischer, 1936
U. nuda	9	refrigerated	Tapke, 1948
Entyloma dahliae	10	herbarium	Fischer, 1936
T. caries	10-18*	ambient	Lowther, 1950; Kendrick and Holton, 1960
T. foetida	10-18*	ambient	Lowther, 1950; Kendrick and Holton, 1960
E. eryngii (Corda) de Bary	11	herbarium	Fischer, 1936
T. holci (Westen.) de Toni (=*T. rauwenhoffii*)	12	herbarium	Fischer, 1936
T. separata Kunze	12	herbarium	Fischer, 1936
Sphacelotheca sorghi	13	herbarium	Fischer & Holton, 1957
T. caries (=*T. tritici*)	13-18*	herbarium	Fischer & Holton, 1957
T. foetida	25	herbarium	Fischer & Holton, 1957
Urocystis colchici	25	soil	Thaxter, 1890
Ustilago crameri	64	herbarium	Wang, 1936

* depending on race and source

5.2.2 Methods

Ustilospore survival under ambient conditions (Trials 5A and 5B)

Trial 5A

A random sample of infected stems with smutted inflorescences was collected from farmers' plots in Kiambu District in April 1998. On the following day, the sample was subdivided into four groups, each containing 100 inflorescences. The groups were allocated to different environmental conditions (two in air and two in soil). The conditions were: *1,* ambient % relative humidity in air; *2,* low % relative humidity in air; *3,* ambient light, temperature and moisture on the surface of the soil; and *4,* ambient temperature and moisture when buried below the soil surface. Inflorescences at condition *1* were tied in a bundle with string and suspended from a beam in a wooden shed. Those at condition *2* were kept indoors in a desiccator over self-indicating silica gel. For conditions *3* and *4*, 200 open ended bags,15cm x 3cm, were made using nylon mesh of 1mm^2 pore size. One smutted inflorescence was inserted into each mesh bag. A rectangular cavity was then dug in garden soil to a depth of 20cm and 100 bags laid at the bottom of the hole. Excavated soil was carefully replaced to cover the bags. The remaining 100 bags were laid on the soil surface.

Trial 5A took place during the Kenyan long rains' season, from March to May. Daily weather records were kept, including maximum and minimum external temperatures, external % relative humidity (measured at 08:00 and 14:00 hours using a wet and dry bulb hygrometer), and rainfall.

Spore germination was assessed at day one, and thereafter at intervals of two weeks. On each sampling occasion, ten inflorescences (ie ten replicates) from each condition were removed from their environment. Each inflorescence was held over the exposed surface of a Petri dish containing 2% tap water agar and tapped gently to dislodge the smut ustilospores. The plates were incubated at room temperature for eight hours and then examined under a microscope for signs of germination. A period of eight hours was long enough to allow ustilospore germination and short enough to prevent overgrowth by saprophytes. Hoy *et al.* (1993) also used an eight hour incubation period in their study on longevity of *U. scitaminea* spores in soil.

Plates were fixed to the moving stage of the microscope with sticky tape and then moved across the visual field. The eyepiece graticule was aligned vertically and every spore that crossed the graticule was assessed, as long as the presence or absence of germination could be distinguished. The percentage ustilospore germination per plate was then calculated. At least 50 ustilospores were counted on each plate, ie over 500 spores per environmental condition and more than 2000 ustilospores per sampling occasion.

On sampling occasions when no germination was observed, plates were re-examined after a further 16 hours, to confirm that the ustilospores were no longer viable.

Trial 5B

To substantiate the results from Trial 5A, and take advantage of a period of drier, cooler weather after the long rains, a second trial was done, commencing mid-July 1998. As well as providing a contrast in environmental conditions, Trial 5B was set up to confirm the longevity interval determined in Trial 5A, since this interval would have a bearing both on the advice given to farmers for disease management, and on the epidemiology of the disease.

The method used in Trial 5A was repeated, except that sampling was limited to two occasions; at the start of the trial and ten weeks later, thus encompassing the viability period established during Trial 5A.

Ustilospore survival at different temperatures

Trial 5C

There were two aspects to this trial. Firstly, to determine the optimum temperature range for ustilospore germination, and secondly to assess whether ustilospores could germinate after storage at low temperatures for different periods of time.

Several infected inflorescences were collected from diseased Napier grass plants. The inflorescences were held over exposed tap water agar in Petri dishes, and gently tapped to release the ustilospores. The dishes were then placed in incubators at a range of temperatures and left for various periods.

The temperatures and incubation times are shown in Table 29. There were six replications at every temperature and time combination. At the end of every incubation period the plates were removed and ustilospore germination determined by microscopic examination, as for Trials 5A and 5B. The baseline germination rate was also measured. To assess the ability of ustilospores to survive low temperatures, plates kept at less than 5°C were incubated at 30°C for 24h and germination measured as before.

Table 29. Temperatures and exposure times to assess survival of ustilospores of *Ustilago kamerunensis.*

temperature (°C)	time of exposure (days)		
-25	1	7	30
-10	1	7	30
0	1	7	30
5	1	7	-[1]
15	1	7	-[1]
20	1	7	-[1]
25	1	-[1]	
35	1	-[1]	
40	1	-[2]	

[1] spore germination obscured by the overgrowth of saprophytes and so no measurement possible.
[2] no spore germination at the shorter exposure time and so no measurement possible.

5.2.3 Results

Trial 5A showed that inflorescences buried in the soil for more than four weeks had decayed completely, while those on the soil surface decomposed after six

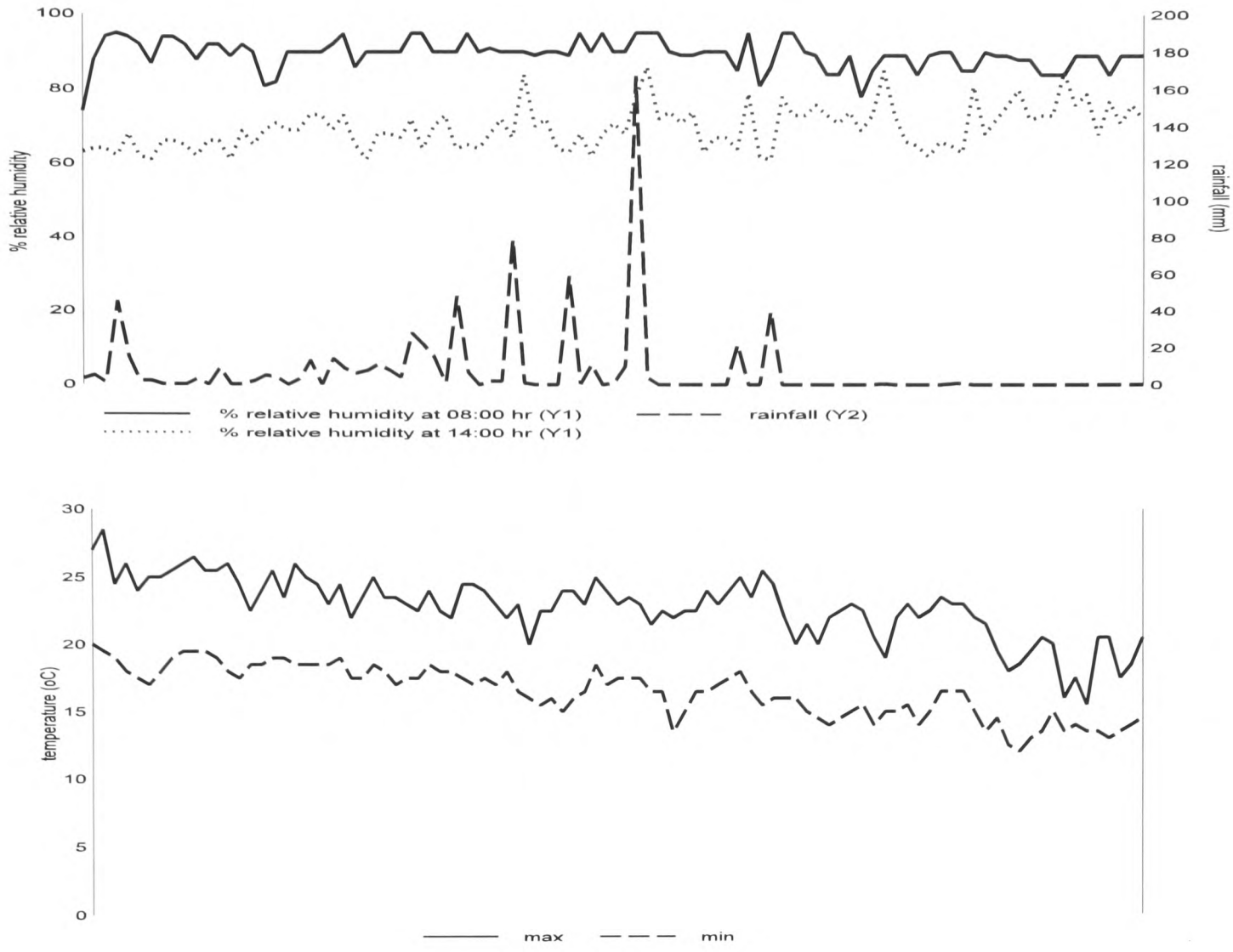
weeks. However, ustilospores could still be seen adhering to soil crumbs, in sufficient numbers to enable assessment of germination ability. It was found that ustilospore germination rates declined quickly, from a maximum of 93.9% at the start of Trial 5A to a complete absence of germination 16 weeks later, and under all conditions. No viable ustilospores in soil could be detected after eight weeks. Air dried spores survived for ten weeks and desiccated spores for 14 weeks, at which time less than one percent were viable. Ustilospore longevity in Trial 5B revealed a similar, declining trend. The germination rate at the start of Trial 5B was 64.5%. After ten weeks there were no viable spores in the buried soil samples, and the mean germination rate of spores from the soil surface was 11.3%. In air dried and desiccated ustilospores, the germination rate was 27%.

Trial 5A ran for 16 weeks, during which the *el Niño* weather phenomenon brought higher than normal rainfall and extended the long rains' season into June. Thereafter, the rains diminished and so the period of Trial 5B, July-September, was dry and cool, as is normal in Nairobi at this time of year. Meteorological conditions during the trials are shown in Table 30. The weather parameters and decline in ustilospore viability over time for Trial 5A are displayed graphically in Figure 19.

Table 30. Monthly meteorological conditions during trials on the longevity of *Ustilago kamerunensis* ustilospores.

month	mean min. temp. (°C)	mean max. temp.(°C)	total rain (mm)	mean %RH 08:00	mean %RH 14:00	18 year average* mean min. temp.(°C)	18 year average* mean max. temp. (°C)	18 year average* total rain (mm)
Trial 5A								
April	18.7	25.1	164	90	66	14.9	24.1	224
May	17.0	23.1	503	91	69	14.1	22.9	173
June	15.3	22.1	64	88	71	12.4	21.5	35
Trial 5B								
July	14.2	19.5	26	88	76	11.4	20.9	23
Aug.	15.2	19.0	22	92	81	11.9	21.6	23
Sept.	15.1	24.7	12	92	77	11.9	23.4	25

* at the National Agricultural Research Laboratories, Nairobi. Relative humidities were not recorded.



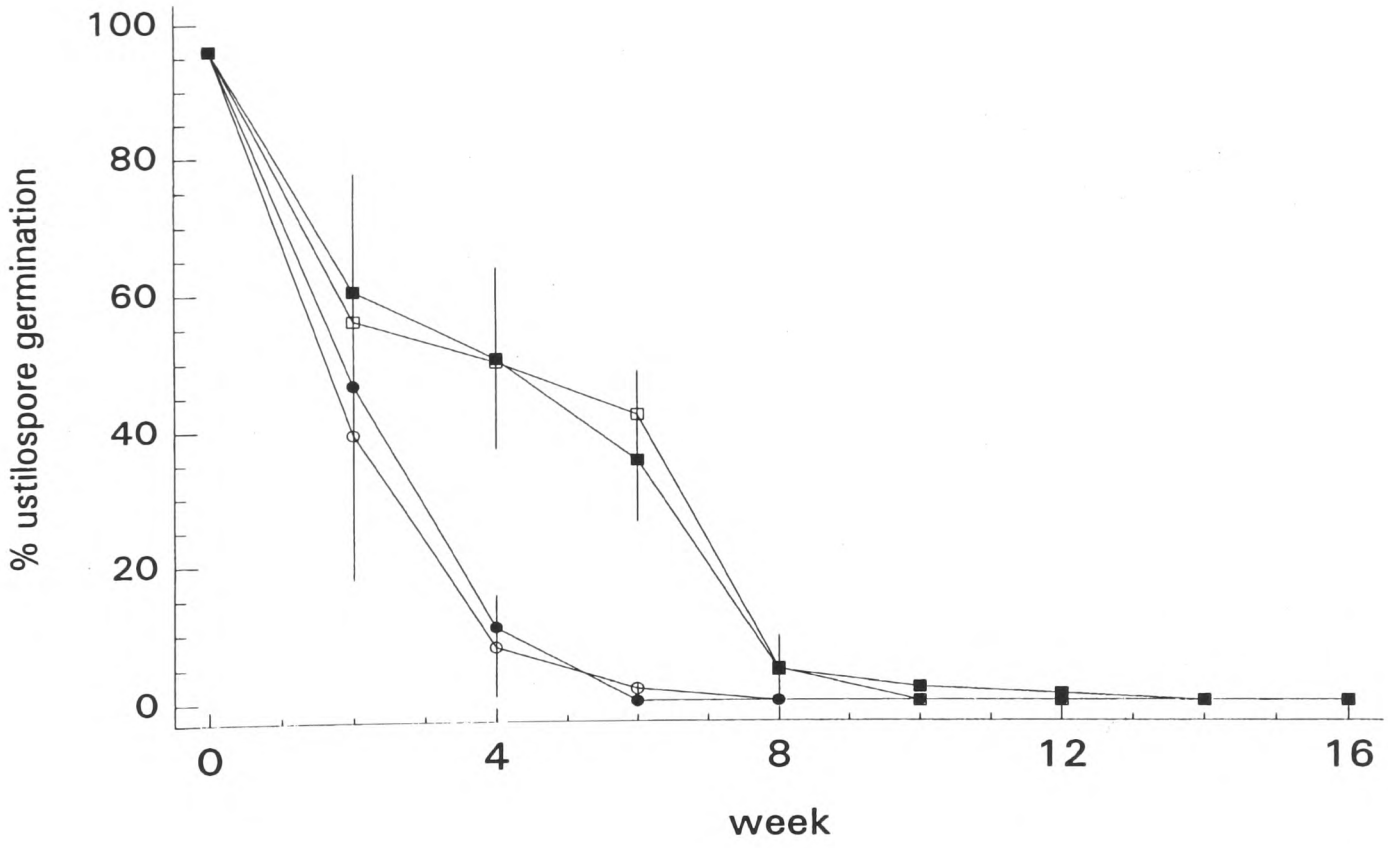


Figure 19. Longevity of ustilospores of *Ustilago kamerunensis* maintained under different environmental conditions (and associated meteorological parameters). ■ = desiccator, □ = air, ● = soil buried, ○ = soil surface. Data points are means of ten replicates and error bars are ± 1 standard deviation (Trial 5A).

In Trial C, ustilopores germinated at a wide range of temperatures above 0°C, as shown in Figure 20. The germination products were sporidia and mycelia at all temperatures. Although there was no ustilospore germination at temperatures of 0°C and below, spores were able to produce sporidia once they had been removed from cold storage and maintained at 30°C for 24h. The germination rates of these spores were in excess of 60 per cent (Table 31), even after storage at -25°C for 30 days.

Table 31. Germination rates of *Ustilago kamerunensis* ustilospores stored at different temperatures.

storage temp. (°C)	% germination rate at that temperature	% germination rate when transferred to 30°C
-25	0	64.5
-10	0	71.2
0	0	75.6
5	25.2	-
15	57.3	-
20	96.7	-
25	86.8	-
35	57.7	-
40	0	-

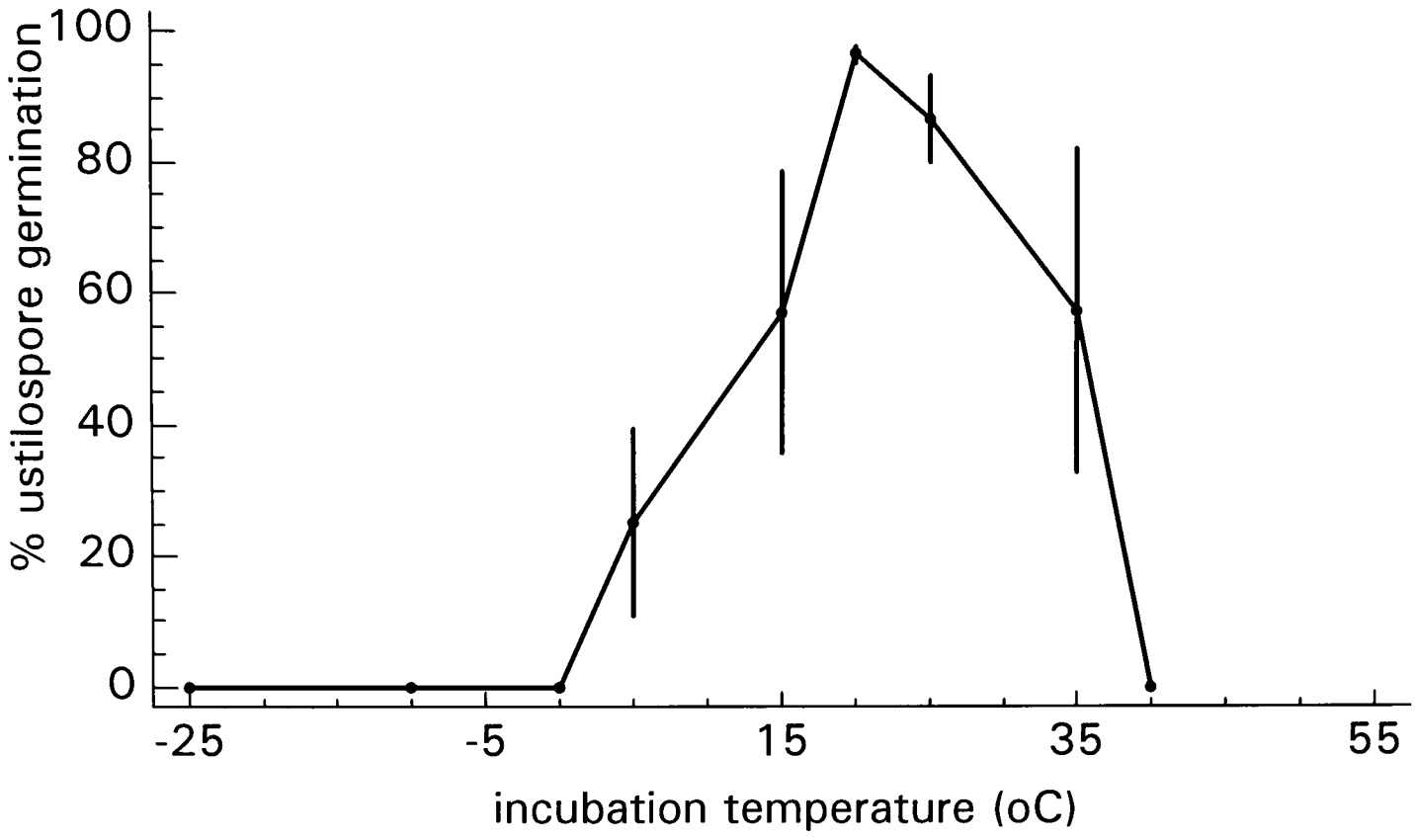


Figure 20. Germination of ustilospores of *Ustilago kamerunensis* after 24h incubation at different temperatures (data points are means of six replicates and error bars are ± 1 standard deviation).

5.2.4 Discussion

Spore germination is a prerequisite to infection in the smut fungi, and is a measure of viability (Holton *et al.*, 1968). Spore longevity varies widely, as was shown in Table 28, and it was in this regard that Fischer and Holton (1957) stressed the influence of environment, as well as genetics, in determining the longevity of smut spores. Evidence is provided by numerous reports of spore longevity from different collections of the same species made at different times of the year, drawn from a variety of hosts, and stored in different ways, all of which show wide variations in spore survival periods

(Holton *et al.,* 1968).

Notwithstanding, Holton *et al.* (1968) pointed out that most reports of longevity are based on incidental observations of herbarium specimens. They concluded that variations in the longevity of smut spores can only be elucidated by assessing spore samples of comparable source and age, under standard conditions. The present investigation suggests that longevity periods of dried specimens are not a good guide to viability under field conditions, particularly for spores which pass through an edaphic phase. Furthermore, a species which germinates quickly in nature may remain dormant for several years when kept in store. Even so, there are examples in which the opposite applies. For instance, Tachibana and Duran (1966) reported that onion smut spores (*Urocystis colchici*) remained viable up to 25 years in soil but were unable to germinate after three years in storage. In the case of *U. kamerunensis*, there was no evidence that ustilospores required a resting period before they were able to germinate, since spores displaced from an inflorescence germinated within eight hours on agar surfaces. However, it is possible that a short resting period could occur while the spores are still attached to their sori on the inflorescence. If *U. kamerunensis* does have a spore dormancy period, it is very short compared with that of most other smut species.

Holton *et al.* (1968) suggested that the incubation period for smut spore germination may be even more variable than the longevity period. Again, this

variation may either reflect the genetic base, or be a simple response to the environment. The incubation period in the smut fungi ranges from a few hours to several weeks, depending on the species. It also appears that the greater the longevity period, the longer the incubation time and the greater its variability in germination (Holton *et al.,* 1968). Hence, *Tilletia* species have a longer incubation period, with higher variability, than *Ustilago* species.

In most cases good spore germination of smut fungi can be obtained at 18° to 20°C (Fischer and Holton, 1957). *Tilletia controversa* is one of the rare exceptions, since this species has an optimum temperature for spore germination of 5°C (Fischer and Holton, 1957). Other published reports of temperature requirements include 5-18°C for *T. caries* and *T. foetida* (Holton *et al.*, 1968), and 15-40°C for *U. scitaminea* (Juangbhanich and Wangwon, 1983). Furthermore, Duran and Safeeulla (1968) established that a chilling period of 60-90 days at 1-2°C was needed to break dormancy in spores of *T. asperifolioides* Fischer.

In this study, ustilospores of *U. kamerunensis* germinated between 5°C and 35°C, with an optimum around 20°C. There would seem, therefore, to be no thermal barrier to spore germination, at the temperatures at which Napier grass thrives in East Africa. In addition, the fact that spores can survive deep freezing for a month, and exhibit germination once thawed, suggests that temperature is not obviously one of the underlying factors in the altitude effect

on smut incidence, insofar as it affects the production of sporidia. However, although optimum temperatures for ustilospore viability were determined, it is not assumed that these temperatures represent optima for infection. The measure of survival used in Trial 5C was the production of sporidia on agar and not infection hyphae or appressoria on plant surfaces. Bock (1964), working with *U. scitaminea* on sugarcane in Kenya, reported temperatures for sporidial production ranging from 24°C to 34°C on potato dextrose agar, but a pronounced optimum temperature of 31°C for the formation of infection hyphae on cane surfaces. An incubation period of two hours was sufficient to produce significant numbers of hyphae at this temperature. In India, with the same pathogen, Juangbhanich and Wangwon (1983) found a similar range of temperatures for ustilospore germination on agar. Nonetheless, the true test of success of spore germination is the subsequent development of infection within the plant, resulting in the production of a smut whip in the case of sugarcane or a smutted inflorescence in Napier grass. Spores, by their nature, are resistant to environmental conditions that would harm more delicate tissues such as hyphae. Low temperatures may therefore contribute to the altitude factor in the spatial distribution of *U. kamerunensis*, not by killing ustilospores but by preventing production or causing the death of germ tubes and appressoria that result from germination on host surfaces.

Light was also considered as an influence, since some smut fungi require it for germination. For instance, germination of spores of *Urocystis occulta* is

stimulated by light (Ling, 1940), but those of *T. caries* are relatively independent (Holton *et al.*, 1968). During these trials, light was not a factor in the germination of *U. kamerunensis* ustilospores, since spores on agar plates in the light and those buried in soil germinated prolifically. Furthermore, and in common with most smut species (Holton *et al.*, 1968), *U. kamerunensis* did not exhibit any nutritional requirements for the initiation of germination.

Trial 5A was carried out during a period of unusually heavy rain, and no viable *U. kamerunensis* spores were found in soil after six weeks. Andreis (1980), when assessing survival of spores of sugarcane smut in Florida soils at different moisture contents, found that spores remained viable in dry soils for about 12 months, but survived less than one week in saturated soil. Andreis (1980) suggested flooding or ploughing sugarcane fields, to expose spores to wet conditions, as a control option. Mansour *et al.* (1991), working with the same pathogen and host in Egypt, reached similar conclusions and also recommended a flooding period before planting. They suggested that spores died because germination occurred in the absence of the host. Although spores of *U. kamerunensis* are similarly susceptible to wet soils, flooding or ploughing up of Napier grass plots could not be recommended as practical management options for subsistence farmers. An added disadvantage is that the grass itself does not withstand waterlogging.

Since Napier grass is fed to zero grazed cattle and the manure is sometimes

used to fertilize Napier grass plots, it is theoretically possible for infection to be spread via this route, providing the ustilospores can survive passage through the alimentary tract. Fischer and Holton (1957) reported conflicting results from other workers of smut spores germinating after extraction from manure, even when they were using the same species of fungus. However, the consensus was that any spores which did survive in animal manure would be very small in number compared with those dispersed by wind, and would therefore be a negligible factor in disease spread. In any case, anecdotal evidence from Kenyan farmers suggests that dairy cattle rejected smutted Napier grass, and so the faecal route is unlikely to play a major role in the spread of *U. kamerunensis*, even assuming that ustilospores could survive passage through the alimentary tract of livestock.

Ustilago kamerunensis effectively exhibits a perennial life cycle in a perennial host. Ustilospore production is continuous and so the fungus does not need to invest resources in producing long lived spores because the Napier grass host is always available. Rather, the fungus puts its energies into producing very large numbers of spores on a continuous basis, thereby continually replenishing the inoculum in the soil.

Although it is reported that livestock densities have remained relatively static over the past decade in Kenya (CBS, 1989, 1996), in fact there is evidence of an increase in the acreage of Napier grass (Staal *et al.*, 1997). During a series

of participatory rural appraisals of 365 households in Kiambu District, 14 percent reported taking up Napier grass cultivation (Staal *et al.*, 1997). Moreover, the increased cutting frequency, which raises the number of germinating tiller buds, increases the number of infection opportunities available to *U. kamerunensis.* These may be factors in the expanding incidence of smut disease of Napier grass over the past decade in Kenya.

5.3 Disease gradients and spore deposition

5.3.1 Introduction

In common with other aspects of the biology of *Ustilago kamerunensis*, there was no information on the epidemiology of the disease it caused. The spread of the fungus in Kenya over the last ten years has been relatively slow, as outlined in Chapter 3. Disease outbreaks have been, for the most part, scattered and not severe, and only rarely has the disease caused the loss of a whole Napier grass stand. Investigations of spore longevity and the infection court suggested that the fungus had only limited opportunities to initiate infection, and that these factors contributed to the pattern of the disease. However, it was considered necessary to attempt further elucidation of the infection process. A better understanding of the spread of the smut disease within a Napier grass stand could also assist in the development of disease management techniques. An experimental plot was therefore established to examine disease gradients and ustilospore dispersal of *U. kamerunensis* (Trial 5D). Gradients were studied using the method of Momol *et al.* (1990) for

sugarcane smut. Spore deposition was assessed through a simple trapping technique.

5.3.2 Methods

Disease gradients

Trial 5D

A plot of Napier grass at the National Agricultural Research Laboratories in Nairobi was established. The land had no history of Napier grass cultivation, having previously been used for experiments on potato wilt disease. The plot was cleared of weeds and 400 Napier grass splits were planted in eight columns, with 50 plants per column, in two blocks of four columns each. A central column was left vacant between the blocks. The columns were 90cm apart and there was a 50cm gap between plants (these were the spacings recommended by MoALDM for Napier grass on farmers' fields). The central column was then planted with smut infected Napier grass of unknown type, to provide a source of disease inoculum. The diseased plants all had smutted inflorescences. Healthy plants were obtained from the museum at KARI Muguga, from a pure stand of the Muguga bana type. Diseased plants were collected from farmers' plots in Lari Division. The planting date for all stools was 28 April 1997.

The plot was regularly inspected. Planting coincided with the beginning of the long rains' season and all the stools established successfully. Growth was

vigorous with the production of many new shoots. However, there was no sign of smut disease in the healthy stools after five months (by October 1997), despite the continuous production of spore-bearing inflorescences from the diseased plants during this period. It was therefore decided to assist the infection process by stressing some of the healthy plants. This was done by cutting them back to ground level, which also encouraged the germination of new tiller buds that previous experiments had shown to be the sites of entry for the fungus. To provide a comparison with uncut stools, only half of the plants were cut. There were five replicates of each cut and uncut block, every replicate consisting of five rows of plants. The diseased plants were left untouched. Figure 21 shows the cutting arrangement.

The extended short rains' season again encouraged lush growth of all the stools. Monitoring of the plot continued but by January 1998 (four more months having passed) there was still no sign of infection, in the cut or uncut blocks. By this time the uncut plants had reached a height of 4m, far exceeding the height of stools normally encountered in farmers' plots and rendering infection even more unlikely, given the maturity of the grass, the impenetrability of the stand and the lack of new tiller production. The management of the plot was therefore changed once more, in a further attempt to induce infection.

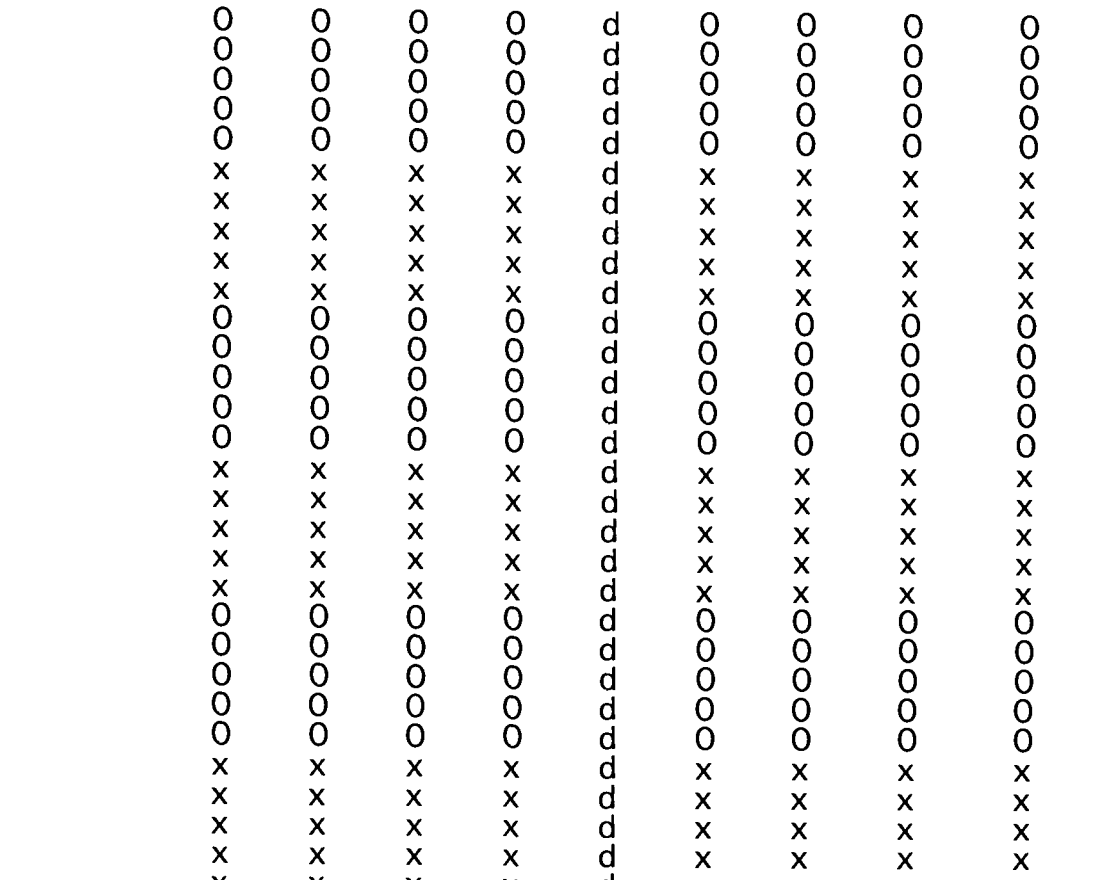


Figure 21. Plan of the epidemiology experiment (Trial 5D). 0 = plant cut, x = plant not cut and d = diseased plant (not to scale).

Henceforth, a more regular cutting regime was employed, in which all of the healthy plants were cut every two months, to more closely duplicate the cutting frequency used by farmers. Cutting was done during the extended long

rains' season of 1998, from February to July. Plant growth was again vigorous, with plentiful tillering and biomass production. The rains stopped in late July, leading to a reduction in the rate of plant growth, and so the last cut was made in that month. By September 1998 no infection was apparent.

The lack of disease spread after 16 months' exposure to an inoculum source was very disappointing, and led to a reappraisal of the rationale for the trial. Various reasons for the inability to induce an epidemic were considered, such as Muguga bana being resistant, unusual environmental conditions (prolonged and heavy rain, waterlogging or low temperatures reducing ustilospore viability) and soil antagonism, possibly influenced by the history of the plot. All of these factors could have had an influence, although observations during the Napier grass disease survey, and information from elsewhere (A Lowe, *pers. comm.*), suggested that Muguga bana was not resistant, and that ustilospores should have survived long enough to enable infection to occur, even in wet soil. However, in setting up the trial the assumption had been made that the mechanisms of ustilospore dispersal and deposition were efficient in distributing viable spores, in sufficient concentrations to induce infection within the plot. To test this hypothesis a spore trapping experiment was set up, using the same stand.

Ustilospore deposition

Trial 5E

The deposition of ustilospores was assessed by trapping spores on horizontal surfaces, making use of gravitational settling. This method was inexpensive and easy to replicate, and provided quantitative data. It may, however, lead to underestimates of spore loads because collection efficiency is subject to the influence of wind and eddies and is selective for larger spores (Gregory and Stedman, 1953). Efficiency can be increased by inclining the trap surface at a 45° angle to the direction of the wind but inclined traps are very sensitive to changes in wind speed, and so it is necessary to record this parameter to enable correction for mean time spore concentration (Gregory and Stedman, 1953). Unfortunately, a recording anemometer was not available and so the traps were laid out horizontally.

In the first instance, the traps were made by covering plastic Petri dish lids with double-sided sticky tape (tape was used rather than flat glass plates, so that, in the event of rain, spores would not be washed off). However, on examination of exposed sticky traps it was discovered that many ustilospores were deposited in clumps, estimated to contain more than 100 individual spores. This made it very difficult to accurately count the individual number of spores deposited, and so the use of sticky tape was abandoned and shallow, circular plastic trays were substituted. These trays had an area of 107.5 cm^2. They were placed downwind from the inoculum source, within and beyond the

Napier grass stand. Trays were left in place for 24h at a time, after which they were exchanged for fresh ones. Traps in the plot were set at ground level beneath the canopy, and at 0.5m high (the height of the healthy Napier grass stools), supported on canes. Those outside the stand were placed on larger trays on the ground. Trap replacement was done on five occasions. There were five smut-infected Napier grass plants in the centre column that provided the ustilospore inoculum. The minimum inoculum-to-trap distance was 4m and the maximum was 20m. Traps were set out during periods of warm, sunny weather, with light winds (estimated at 1.7 to 3.1ms^{-1}) blowing from the South-East. Figure 22 shows the layout of the plot and disposition of the traps.

After collection of the traps, the ustilospores were washed off with a known volume of water. Spores were counted using a haemocytometer, and the number of ustilospores deposited per cm^2 per day was calculated.

It was assumed that there was a constant proportion of viable ustilospores in the spore catch. To test this hypothesis spores were also trapped on exposed tap water agar surfaces . The ustilospores were examined microscopically and the percentage ustilospore germination rate was calculated. Propagule viability is usually assessed by exposing culture media to airborne spores (Campbell and Madden, 1990). However, use of a selective medium is desirable, to limit overgrowth by saprophytes during the exposure period. Such a medium has

not been developed for *U. kamerunensis.* Even so, an exposure period of 24h was found to be long enough to trap *U. kamerunensis* spores, and assess their viability, without the plates being swamped by overgrowth.

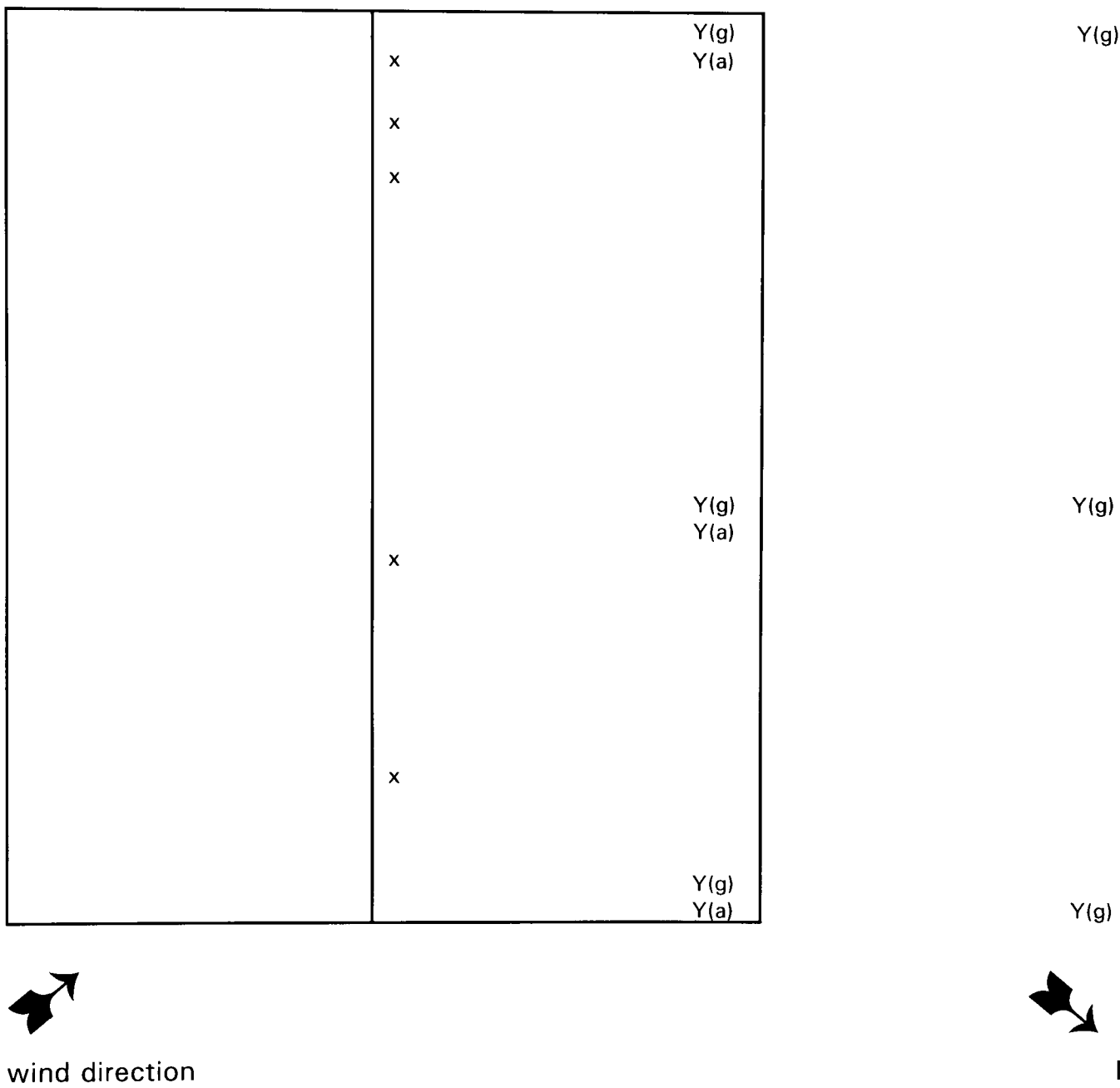


Figure 22. Layout of the Napier grass stand and sites of ustilospore traps (not to scale). x = smut infected Napier grass stool; Y(g) = trap on ground; Y(a) = trap above ground.

5.3.3 Results

Ustilospore catches were highly variable, with deposition rates ranging from 20-50 spores per cm^2 per day in traps at 0.5m high in the canopy (4m from the

inoculum source), and in traps 20m distant at ground level. Few spores were caught at ground level within the stand, presumably because of the closure of the canopy that limited air movements beneath the leaves. Close examination of plants near the inoculum sources revealed many spores on the upper surfaces of leaves and in leaf axils, presumably washed there by rain. The numbers of trapped ustilospores and their germination rates are shown in Table 32.

Table 32. Catches (number of spores per cm^2 per day) and germination rates of ustilospores of *Ustilago kamerunensis* trapped in a stand of Napier grass.

	traps within stand		traps outside stand
trap height	0.5m	ground level	ground level
spore catch[1]	43.0	0.6	14.6
% germination rate of captured spores	63.8	62.8	61.6

[1] means of five replicates

5.3.4 Discussion

It is difficult to assess the deposition of ustilospores at ground level within the crop using this trapping methodology. Spores deposited on leaf surfaces and beneath leaf sheaths may contribute greatly to the soil spore load if heavy rain washes them off plant surfaces, though they may have lost much of their viability by the time they reach the soil, depending on the length of time the

spores were lodged in the canopy. The soil spore-load may be low when ustilospores are trapped on leaf surfaces, but this may not be important in reducing infection pressure because tiller bud elongation is much reduced when the leaves are growing. It is only when the leaves are removed by cutting that the tiller buds push through the soil and become susceptible to infection, and at this time there are no leaves present to catch falling spores.

A further complication is that rain will hinder the release of spores from the plant, as many adhere to the inflorescence when made wet. Ustilospores of *U. kamerunensis* were not shed rapidly from the inflorescence, being dispersed over a period of 7-10 days (depending on wind speed) from the first emergence of the smutted inflorescence. With sugarcane smut, Waller (1969) reported that few spores remained on smut whips that had been exposed for two days.

Despite these reservations it is clear that ustilospore catches were low, compared with reports from other workers. For example, Waller (1969) reported trapping up to 1000 spores of *U. scitaminea* per cm^2 per day, 10m downwind of the inoculum source, though Waller also found very variable deposition, with few spores reaching the soil beneath the sugarcane crop. Campbell and Madden (1990) point out that horizontal surface traps that rely on settling or impaction are selective for larger spores, and, since *U. kamerunensis* spores are at the smaller end of the range for smuts, being 6-

8μm in diameter, it is likely that the results presented here are underestimates. However, it was noteworthy that many of the spores observed on the sticky traps and agar surfaces were in clumps, and so dispersal from smutted inflorescences is not just by single spores in this species.

The viability of trapped spores was high (Table 32), suggesting that lack of an epidemic within the trial plot was not due to poor ustilospore germination rates. Although the supposition was not quantified, it was interesting to note that spores appeared to have a lower germination rate when they remained in large clumps, compared to those observed singly or in small groups of less than ten. It is proposed that some inhibitory mechanism operates, which prevents premature germination when mature ustilospores are still attached to the host, This mechanism breaks down once the spores are released and disaggregated (by the action of water in the soil), to maximise the number of spores that are potentially infectious. Diffusible pheromones have been identified in some Basidiomycetes that mediate cell fusion (Anderson *et al.,* 1992; Bolker and Kahmann, 1993), and it may be that pheromones are active in preventing germination of contiguous ustilospores in *U. kamerunensis*.

In conclusion, it appears likely that the reason for the lack of an epidemic in the trial plot was the low number of spores reaching the soil surface, together with the limited availability of infection niches.

CHAPTER 6 REDUCTION IN BIOMASS IN SMUT-INFECTED NAPIER GRASS

6.1 Introduction

The development of integrated pest management as a paradigm in crop protection has traditionally concentrated on the problem solving aspects of yield maximisation. More recently, increasing attention has been paid to the problem definition constituent, namely crop loss assessment. This has come about because reliable information on crop losses caused by pests and diseases is needed to establish the yield increases obtainable when these organisms are managed at an acceptable economic cost (Teng, 1987). The benefits to be gained from plant protection measures can then be demonstrated to growers and consumers (Chiarappa, 1971). In addition, crop loss information enables researchers and policy makers to determine needs and priorities when planning interventions, and to justify such interventions to funding agencies. To develop a rational and economical plant protection programme it is therefore essential to obtain reliable crop loss estimates; *"... the cost of loss must be known so that it can be compared with the cost of control."* (James, 1983).

Assessing crop losses caused by plant pathogens usually involves collection of two types of data; *disease incidence* (counting the number of affected plants, and expressing the number as a percentage of the total number of plants in the field) and percent *disease severity* (dividing the area of diseased plant tissue by the total area, and multiplying by 100). Pictorial assessment keys are often

used to measure disease severity in the field (James, 1971a, 1971b). When appraising incidence and severity it is also important to include an estimate of the growth stage of the crop, since this influences disease susceptibility and the type of disease likely to be observed.

Disease incidence is frequently employed for assessing systemic infections, for example wilts or viruses, or when the diseased plants or plant parts are totally lost, as is the case with cereal bunts and smuts (James, 1983). However, measuring disease incidence in pasture or fodder crops can be difficult because it is sometimes impossible to distinguish individual plants. Researchers usually rely on assessments of biomass reduction, expressed as a loss in kilograms of wet or dry weight per unit area. Other methods involve assessment of quality losses, modelling, measuring changes in species composition and grazing trials (Lenné, 1989).

Various methods of measuring smut disease incidence and yield reduction have been proposed, and these are summarised, for cereals and sugarcane, in Table 33. Most of the studies on estimates of losses due to smuts in forage grasses are from temperate areas (J M Lenné, *pers. comm.*), though Dawar and Singh (1975) have estimated the loss in gross production of the fodder grass, *Iseilema laxum* Hack, infected by *Sphacelotheca inayati* (=*Sporisorium inayati* (Sydow and Sydow and Butler) Vánky), as 61 percent. For temperate grasses, Falloon *et al*. (1988), working in New Zealand, measured dry matter biomass

reductions of 30 percent in prairie grass (*Bromus willdenowii*) infected by *U. bullata*, that increased to 47 percent biomass reduction when comparing completely healthy and completely infected swards (Falloon and Hume, 1988). There have been no attempts to measure disease severity for smut infections, because most work has been done on cereal crops in which a smutted head is counted as a total loss.

The growth pattern and habit of smut infected Napier grass provide opportunities for the development of a novel assessment method for biomass reduction in a tropical fodder crop. Firstly, the plants are discrete and so they can be assessed individually and, secondly, the disease can be more or less systemic, opening the way to estimations of disease severity per infected plant (since an infected plant may not necessarily be totally valueless). Finally, there are no other serious pests or diseases of Napier grass at present, and so loss in yield can be confidently ascribed solely to *U. kamerunensis.* An attempt was therefore made to develop a sampling regime, for use in field situations, that was designed to measure the fresh weight biomass reduction of Napier grass infected by *U. kamerunensis*.

Table 33. Methods of estimating crop yield reductions caused by smut fungi, and disease incidence.

crop	pathogen	common name	method	reference
sorghum[1]	*Sphacelotheca cruenta*	loose smut	score disease incidence[2]	Sutherland *et al.*, 1996
	S. sorghi	covered smut	score disease incidence[2] sample single sprigs[3] weigh cobs[4]	Sutherland *et al.*, 1996 Harris, 1963 Mathur *et al.*, 1965
	Tolyposporium (= *Sorosporium*) *ehrenbergii*	long smut	score disease incidence[2]	Sutherland *et al.*, 1996
	S. reiliana	head smut	record as present or absent	Sutherland *et al.*, 1996
maize	*S. reiliana*	head smut	record as present or absent	Sutherland *et al.*, 1996
	Ustilago maydis	common smut	record as present or absent	Sutherland *et al.*, 1996
wheat	*U. nuda*	loose smut	record proportion of ears affected	Sutherland *et al.*, 1996
	Tilletia spp.	bunt	record proportion of ears affected	Sutherland *et al.*, 1996; Slinkard & Elliot, 1954
wheat, barley, oats	*Tilletia* and *Ustilago* spp.	bunt, loose smut	record proportion of ears affected[5]	Kagan & Studziński, 1967
sugarcane	*U. scitaminea*	smut	score disease incidence[6] compare yields from healthy and diseased canes	Sutherland *et al.*, 1996 Whittle, 1982; Glaz *et al.*, 1989;

[1]Record at heading stage (post-flowering).

[2] **0** = no disease; **1** = 1-10% of head affected; **2** = 11-30% of head affected; **3** = >30% of head affected.

[3]Take single springs from top, middle and bottom of successive panicles. From sprigs select 500 spikelets and determine percentage infected (50 spikelets from 500 sprigs/field is adequate). The percentage of infected spikelets is directly proportional to the loss of grain yield.

[4]Weigh healthy and diseased cobs and assess percentage infection per cob. Then correlate percentage infection per cob and percentage infection per area with loss of grain yield.

[5]Select 50 plants from five sites of $1m^2$/transect. Calculate percentage of infected ears using:

$$\text{loss} = \frac{Pr}{100\text{-}Pr} \times Pa$$

where Pr = percentage infected ears and Pa = actual yield (kg/ha).

[6]Score as proportion of stems affected in sampled plants on scale of **0** to **3**.

6.2 Methods

Data collection

Development of the sampling regime involved the examination of stools of smut-infected Napier grass, assigning a damage score to each stool and then cutting and measuring the fresh weight of the stool. From previous field observations, five damage classes were considered sufficient to contain the range of damage normally seen in infected Napier grass plots. Damage classes were based on the approximate percentage of the stool that showed signs of smut disease. Thus stools given class *1* were free of smut infection, class *2* stools were less than 25 percent smutted, class *3* were 25-50 percent smutted, class *4* were 50-75 percent smutted and class *5* were 75-100 percent smutted. A schematic representation of the damage classes is shown in Figure 23.

Heavily infected plots were chosen to provide a full range of smut infection. To give sufficient degrees of freedom, 10-15 plots had to be sampled (R Coe, *pers. comm.*). Where possible, at least ten plants were assessed from each damage class per plot. Every plant was assigned to the appropriate class and then cut at ground level (the normal harvesting technique) and weighed to the nearest 100g using a spring balance suspended on a tripod. The average height of the plants per plot was also recorded.

Class 1
healthy

Class 2
<25%

Class 3
25-50%

Class 4
50-75%

Class 5
>75%

Figure 23. Schematic diagrams of damage class and the percentage of the Napier grass stool affected by *Ustilago kamerunensis*.

Data analysis

The aim of the exercise was to develop a sampling regime and guidelines for calculation of biomass reduction that would be applicable to all Napier grass plots. However, the data set incorporated fixed and random effects, in that there was variation within plots (from the damage class variable), and between plots. The random effects comprised differences between plots, plant weights and plot by class interaction. The fixed effect was provided by the class variable. For these reasons, the data were subjected to residual maximum likelihood (REML) analysis. The REML method was chosen because, unlike regression analysis, it can account for more than one source of variation in the data and provide an estimate of the variance components associated with the random terms in a linear mixed model. Its use thus allows assessment of the relative importance of the sources of variability (Payne *et al.*, 1987). This method will also show if a model of damage class and weight can be constructed and if one formula for calculation of biomass reduction can be used for assessing diseased Napier grass plots of different heights. The variables subjected to REML analysis were plot number, stool weight, damage class x plot number and damage class x height of stand.

6.3 Results

Data collection

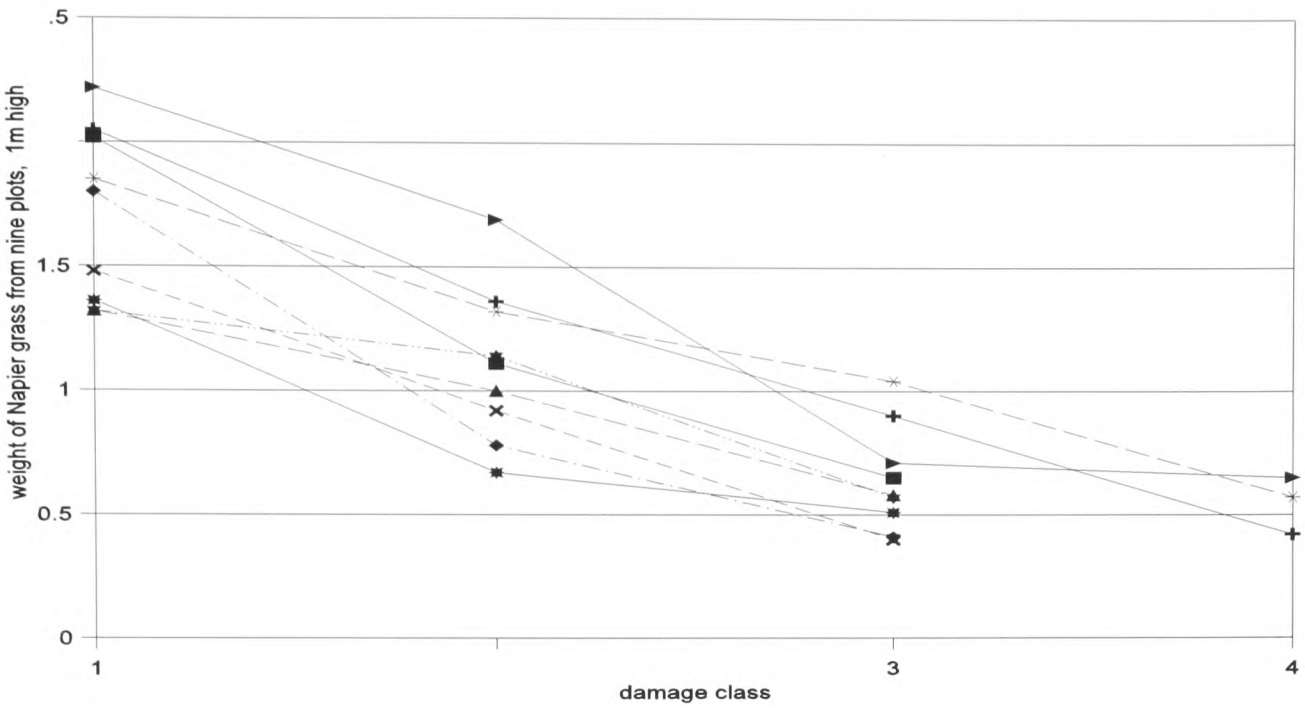
Fourteen Napier grass plots in Lari Division, Kiambu District, were assessed for the severity of infection by *U. kamerunensis.* Nine plots were 1m high and five

were 1.5m (the MoALDM recommend that Napier grass be cut at 1-1.5m for feeding to livestock). Four hundred and forty eight stools were measured and all damage classes were seen. However, only two plants were assigned to damage class *5,* and so these were removed from the data set.

Data analysis

The range of fresh weights at every damage class is shown in Figure 24, for stands of Napier grass at 1m and 1.5m in height. Before analysis by REML, stool weights were transformed to log values to give the data sets a more normal frequency distribution. Heights of the stands were incorporated into the test to determine whether one sampling protocol would fit stands of differing heights. The results of the REML variance components analysis for all plants, shown in Table 34, indicate that stool weight provides the largest source of error in the model.

A)



B)

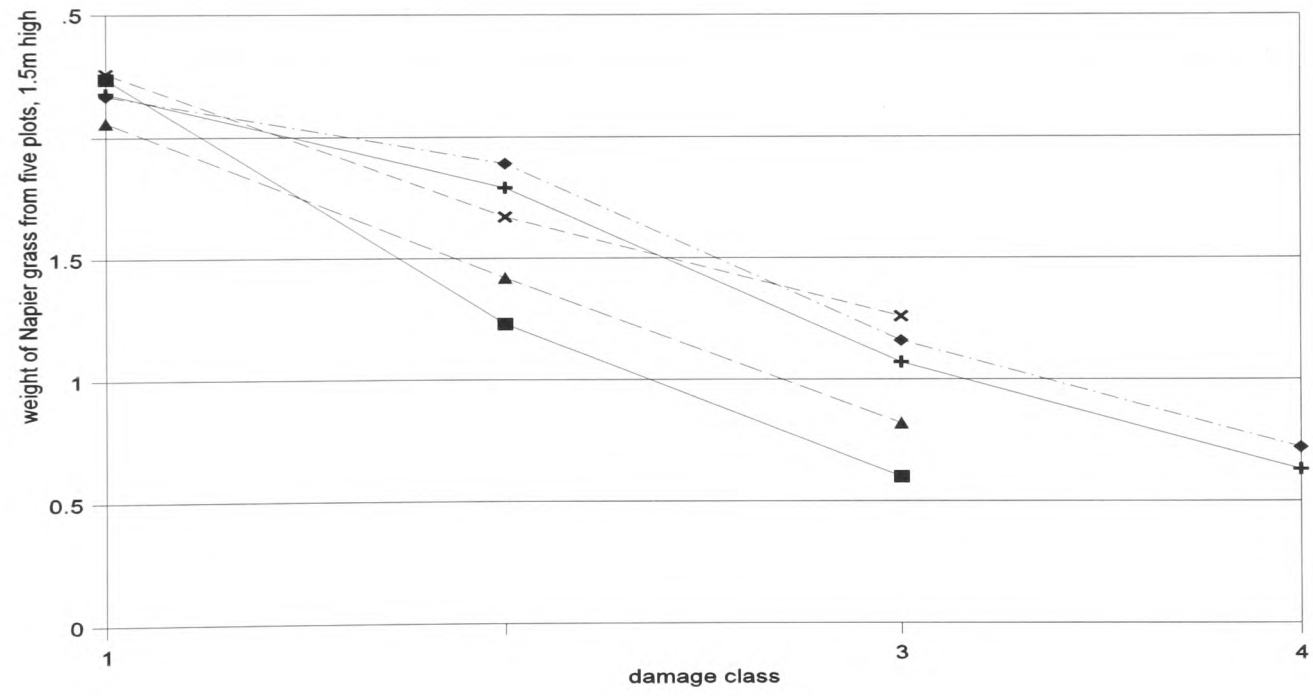


Figure 24. Graphical representation of the relationship between fresh weight of smut-infected Napier grass stools and associated damage class, in stands 1m high (A) and 1.5m high (B).

Table 34. Estimates of variance components, using residual maximum likelihood analysis, of 448 Napier grass plant weights (log transformed) and damage class from 14 smut-infected plots.

	variance	se
random effects		
plots	0.0383	0.0194
plots x class	0.0082	0.0078
weight	0.1881	0.0133

	Wald statistic*	df	*p*
fixed effects			
class	338.5	3	<0.005
class x height	11.1	4	>0.01

* The Wald statistic is used to test null hypotheses for fixed term models, and is calculated by dividing the treatment sum of squares by the population variance. It has an asymptotic χ^2 distribution with degrees of freedom equal to those of the model term (Payne *et al.*, 1987).

A value of 11.1 for the Wald statistic of class x height ($0.025>p>0.01$) suggests that height effects are marginally significant and therefore one model cannot be used to describe stands of differing heights. Because of this the data for the different heights were analysed separately, and Table 35 shows the results of this analysis. It can be seen from Table 36 that plants with less than 25 percent infection, as represented by damage class 2, can still suffer a biomass loss of 26-35 percent.

Table 35. Estimates of variance components, using residual maximum likelihood analysis, of Napier grass plant weights (log transformed) and damage class from stands of different heights.

	stand height (and number of stools)			
effect	1m (284) variance	se	1.5m (164) variance	se
random effects				
plots	0.0471	0.029	0.021	0.0197
plot x class	0.0103	0.0108	0.0039	0.0102
weight	0.2003	0.0178	0.1671	0.0195

	Wald statistic	df	*p*	Wald statistic	df	*p*
fixed effect						
class	210.1	3	<0.005	139.1	3	<0.005

Table 36. Comparison of relative stool weights, percent biomass reduction and damage class from smutted Napier grass stands at different heights.

damage class	relative stool weights and % biomass reduction					
	at 1m	% biomass reduction	theoretical maximum[1]	at 1.5m	% biomass reduction	theoretical maximum[1]
1	1.000	0	0	1.000	0	0
2	0.648	35	25	0.739	26	25
3	0.368	63	50	0.426	57	50
4	0.233	77	75	0.282	72	75

[1] where class 2 represents up to 25% loss, class 3 represents 25-50% loss and class 4 represents 50-75% loss.

The hypothesis that damage class is correlated with stool weight is confirmed by the significant values obtained for the Wald statistic for class, as shown in Table 34. A formula for the calculation of biomass reduction can therefore be

derived by summing the number of plants in each damage class, and multiplying by the corresponding weights from Table 36, for each height. The formulae for stands of different height are shown in Figure 25.

For stands 1m high, the total % biomass reduction =

$$100 \times \left(\frac{n_2}{n_1}\right) \times (1 - 0.648) + \left(\frac{n_3}{n_1}\right) \times (1 - 0.368) + \left(\frac{n_4}{n_1}\right) \times (1 - 0.2333)$$

For stands 1.5m high, the total % biomass reduction =

$$100 \times \left(\frac{n_2}{n_1}\right) \times (1 - 0.739) + \left(\frac{n_3}{n_1}\right) \times (1 - 0.426) + \left(\frac{n_4}{n_1}\right) \times (1 - 0.282)$$

where n_1 = total number of stools, n_2 = number of stools in damage class *2*, n_3 = number of stools in damage class *3*, n_4 = number of stools in damage class *4*.

Figure 25. Equations for the calculation of percent biomass reduction in Napier grass infected with *Ustilago kamerunensis*.

Calculation of biomass reduction can be simplified by setting up a template using a computer spreadsheet programme. Table 37 shows such a template, and gives two examples of biomass reduction for stands at 1 and 1.5m. Additional examples are given in Table 38, which includes a comparison with biomass reductions if a diseased plant is destroyed and is therefore considered to be a total loss.

Table 37. A spreadsheet template to calculate total biomass reduction and error values for Napier grass infected by *Ustilago kamerunensis,* with examples.

Data for 1m stands.			class				Total
			1	2	3	4	
model	log	log weight	0	-0.4337	-0.9995	-1.4553	
model		weight	1	0.6481067	0.3680634	0.2333304	
model	log	sed of log weight	0	0.1076	0.1076	0.1076	
model	log	variance of plot X class	0	0.0103	0.0103	0.0103	
model	log	variance per plant	0	0.2003	0.2003	0.2003	
sample		number	100	40	20	20	180
sample		fraction	0.555556	0.222222	0.111111	0.111111	
sample		reduction	0	0.351893	0.631937	0.76667	
sample		weighted reduction	0	0.0781985	0.0702152	0.0851855	0.2335992
sample		variance of log weight	0	0.02689	0.03189	0.03189	
sample		variance of weight	0	0.09828	0.06659	0.05086	
sample		variance of weighted reduction	0	0.0048532	0.0008221	0.0006279	0.0063
sample		se of weighted reduction					0.0793922
Data for 1.5m stands			class				Total
			1	2	3	4	
model	log	log weight	0	-0.3027	-0.8535	-1.2669	
model		weight	1	0.738821	0.425922	0.281704	
model	log	sed of log weight	0	0.1138	0.1138	0.1138	
model	log	variance of plot X class	0	0.0039	0.0039	0.0039	
model	log	variance per plant	0	0.1671	0.1671	0.1671	
sample		number	40	10	5	2	57
sample		fraction	0.701754	0.175439	0.08772	0.03509	
sample		reduction	0	0.261179	0.574078	0.718296	
sample		weighted reduction	0	0.0458209	0.0503577	0.0252034	0.121382
sample		variance of log weight	0	0.0335604	0.0502704	0.1004004	
sample		variance of weight	0	0.147082	0.1178322	0.1763683	
sample		variance of weighted reduction	0	0.004527	0.0009067	0.0002171	0.0056508
sample		se of weighted reduction					0.0751719

Table 38. Examples of biomass reduction caused by *Ustilago kamerunensis* infection of Napier grass.

stand height (m)	number of plants in class 1	2	3	4	total % biomass reduction	se	se min.[1]	% reduction if diseased plant is a total loss[2]
1	36	22	10	6	25.2	11.0	8.9	51.4
1	5	33	7	1	36.5	23.5	18.1	89.1
1	19	25	8	1	27.5	16.3	12.9	64.2
1	19	14	19	1	33.9	13.4	10.9	64.2
1	12	9	20	10	46.0	13.4	10.9	75.0
1.5	14	13	14	14	39.1	12.2	9.1	74.5
1.5	12	18	17	5	34.7	14.6	10.5	76.9

[1] if the model was derived from an infinite number of plots.
[2] ie the farmer discards diseased plants without salvaging the healthy portions.

There are three sources of error in the development and use of the model, arising from differences in plant weight within and between plots, and in comparing the model weights with weights in a sample plot (MW/SW). It can be seen from Table 38 that these error components are quite high. The errors can be reduced and Table 38 also indicates the minimum values that can be obtained, if the model is developed from sampling an infinite number of plots. In such a case, the variance of MW/SW (ie the se of the difference of the log weights) will be zero. This is the minimum value possible for the standard error of the total biomass reduction (TBR). When using the method to assess new plots, errors need to be kept to an acceptable minimum, and Figure 26 shows curves of sample size (assuming equal numbers of plants in every damage class), plotted against the standard error of the TBR. It suggests that a sample size of about 60-80 plants is needed per plot, in order to provide a reasonable value for the standard error.

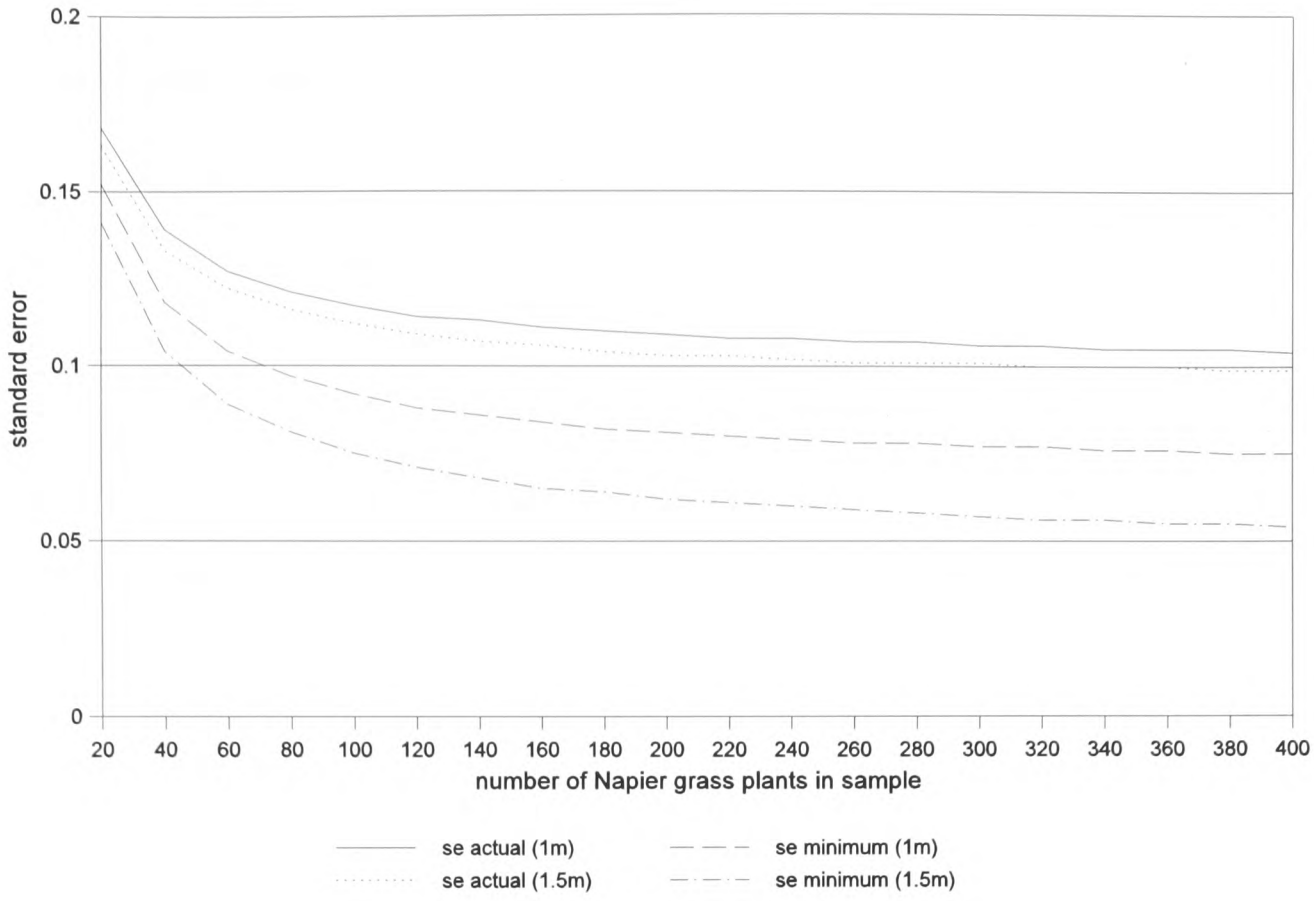


Figure 26. Model (actual) values and minimum values of standard errors plotted against number of Napier grass plants.

The damage class scale described assumes that the healthy parts of smut-infected plants will be salvaged and fed to cattle. If a damaged plant is considered by the farmer to be a total loss than the percentage biomass reduction increases to between 50 and 90 percent, in the examples given in Table 36.

Sampling method for new plots

To use the sampling method, a transect should be constructed across the Napier grass plot and at least 60 stools assessed. Because the distribution of smutted Napier grass plants within a plot tends to be disaggregated, transects

may be simple diagonals (Shepherd and Ferrer, 1990). The number of plants in each damage class are counted and the numbers are fitted into the relevant formula, given in Figure 25, depending on the height of the crop.

6.4 Discussion

"*There is an urgent need to establish simple diagnostic methods to provide accurate data on the effect of individual diseases and on pasture ... production.*" (Lenné and Trutmann, 1994).

Above all, a disease assessment method must be reliable and reproducible (Jones, 1987), and preferably rapid, to encourage its use as a field tool. Methods of recording disease began by assigning subjective, descriptive categories such as *slight, moderate* or *severe* to diseased plants. These categories have been developed to increase the accuracy and reproducibility of disease assessment, an example of which is the nine point scale used to estimate the percentage infection of late blight of potato in the UK (Anon., 1947). Many other assessment keys have since been produced. However, lack of standardisation is a major drawback with descriptive keys, making it impossible to collate results from different observers and compare corresponding data from other researchers (James, 1983). To overcome these difficulties, standardised, pictorial keys (standard area diagrams) have been developed, since they have the advantage of providing qualitative data and reducing estimation errors. Nonetheless, such keys are not applicable to all

host/pathogen interactions, and they are more applicable to foliar diseases. James (1971a and b) and Chiarappa (1971) provide many examples of standard area diagrams.

Unfortunately, pictorial area diagram keys are of limited use for systemic smut diseases. The method described here for *U. kamerunensis* on Napier grass is descriptive and based on a qualitative assessment of the percentage of the plant that is diseased. Even so, the damage scales are relatively straightforward to assign, relying as they do on allocating a simple percentage of the amount of the plant affected. The method also measures that part of the plant that makes the most important contribution to yield ie the stems and leaves.

Biomass reduction in fodder and pasture crops is usually expressed in terms of dry matter weight, whereas the method described above uses fresh weight (freshly cut Napier grass is approximately 20 percent dry matter (Staal *et al.*, 1997)). This was done for ease of development of the model, since the logistics involved in carrying several hundred kilograms of Napier grass from the field to drying ovens in the laboratory were seen as prohibitively costly and time consuming. It could be argued that dry matter determination should be done, to allow comparison with published data on Napier grass yields and to remove errors resulting from the drier weight of smutted stems compared to healthy ones. Nevertheless, the trend in biomass reduction would be the same

whether fresh or dry weight was used, as would be the figure for percentage reduction. Errors from different weights of healthy and diseased stems are likely to be small in comparison with other errors implicit in the sampling method.

When constructing the REML model, it was found that there were too few examples of plants that were scored in damage class 5 (where all of the stool was infected) to merit their inclusion. This was probably due to the fact that the stands were relatively young, as evidenced by their low heights. In the field, however, such plants will be occasionally observed, but they will be so severely smutted that they represent a total loss, having no harvestable portion and minimal food value.

As Lenné and Trutmann (1994) pointed out, evaluation of economic losses to pasture and fodder production is more complicated than for crops because the primary production unit is the animal and not the plant. Sufficient feed may remain on the farm even if large amounts are destroyed by disease, particularly when stock levels are low. In the high potential areas of Kenya, where good rainfall is the norm, the growth of Napier grass is vigorous and it withstands frequent cutting. Under such conditions, availability of the grass is good and losses caused by disease are unlikely to have an appreciable effect on animal production, since the disease is not serious enough to wipe out the crop. However, when rainfall is poor (as was seen in 1997 during the survey

described in Chapter 3), there was evidence that the grass was cut too often to enable good recovery before the next cut. In these circumstances, Napier grass may also be more susceptible to *U. kamerunensis*, further reducing yield. The situation is also complicated by the fact that some farmers sell Napier grass as a cash crop. For these reasons biomass reduction effects on animal production, and farm incomes, cannot be ruled out, despite the large area given over to Napier grass in the high potential areas of Kenya. This was supported by data from a series of participatory appraisals of farmers in Kiambu and Thika Districts in 1998. Some farmers sold their cattle because of lack of Napier grass resulting from smut infection. Yields were reduced from ten bags to two or three bags per plot. Other farmers reported a loss in milk production of 30 percent (Musembi and Nyanyu, 1998).

The loss figures derived from use of the model are the minima likely to be encountered, because they assume that the healthy parts of smut-infected plants can still be fed to livestock. Farmers in Kiambu are reluctant to feed smutted plants to cattle, claiming that the animals begin coughing and reject diseased material (Anon., 1998). However, animal feeding behaviour is subject to many variables, such as the choice of fodders on offer, competition with other cattle feeding from the same trough, and their state of hunger. All of these may leave cattle with no choice but to consume smutted Napier grass. It therefore seems reasonable to assume that actual biomass reduction levels lie somewhere between the minima given by the model and the maxima

resulting from a total loss of diseased plants.

As well as lowering biomass, fungal diseases may also reduce forage quality by depleting protein and amino acid content, decreasing water soluble carbohydrates and diminishing *in vitro* dry matter digestibility (Lenné, 1989). Small changes in these factors could have a major impact on feed value (J M Lenné, *pers. comm.*). Depletion in the feed value of diseased Napier grass is probably unimportant compared with the large losses in biomass already apparent. The challenge also remains in translating decreases in forage quality into a reduction in animal productivity, with the additional complication that animals may refuse to eat diseased forage (Lenné, 1989), as appears to be the case with smut-infected Napier grass. Nonetheless, comparisons of feed values from healthy and diseased stools could be valuable in the interpretation of the effects of *U. kamerunensis* on animal production. There is also a need for a better appreciation of farmers' views on the disease , its effect on their livelihoods and their coping strategies, which could be fed into new disease management technologies. Some preliminary socio-economic evaluation was done by Musembi and Nyanyu (1998) but this needs to be expanded.

The biomass reduction method given above is a preliminary attempt to quantify losses of a tropical fodder crop in a field situation. The method is limited by the time and place of its development, and no claims are made as to its applicability in other countries which may have different Napier grass cropping

patterns or types. However, it is suggested that it may serve as a model to stimulate interest in a neglected aspect of crop loss assessment; a methodology that could become more relevant if the Kenyan smut disease epidemic spreads to neighbouring countries in East Africa.

CHAPTER 7 RESISTANCE OF NAPIER AND OTHER GRASSES TO INFECTION BY *USTILAGO KAMERUNENSIS*

7.1 Introduction

The most economic strategy for managing diseases of tropical pasture plants involves the use of disease resistant germplasm (Lenné and Sonoda, 1990). This is especially true in the case of Napier grass and *U. kamerunensis*, since farmers threatened by the disease are poor in resources. In addition, the grass has traditionally been grown with low or no inputs, and so farmers could be reluctant to use management options that involve additional costs. Resistant or tolerant cultivars have an added advantage compared, for example, with the development of fungicides, in that they can be tested on farmers' fields, with the active cooperation of farmers. Generally, however, resistant or tolerant varieties can only be identified if techniques for creating infection can be standardised (Warham, 1990). Screening for disease resistance in the field, and relying on natural infections, is dependent on optimal environmental conditions for disease development. The host also needs to be in a susceptible state for the pathogen to gain a hold, and this cannot be guaranteed in the natural environment. For these reasons there have been many attempts to develop artificial inoculation methods for plant diseases, that can be used to assess large numbers of lines for disease resistance.

Artificial inoculation has also been used to overcome external host plant

resistance mechanisms, as a means of studying the ways in which plants protect themselves from invasion by smut fungi. Most work has concentrated on sugarcane smut and Karnal bunt of wheat. Table 39 summarises some methods for inducing artificial inoculation. These methods were all employed to assess varietal resistance to smuts or bunt in wheat, barley and sugarcane, and the different methods were compared by the authors for ease of use and maximisation of disease expression.

For the purposes of this study, two resistance testing trials were set up. The dipping method has been most commonly used for initiating smut infections because of its simplicity and cheapness and for inducing high rates of infection. Dipping was therefore adopted in Trial 7A to investigate resistance or tolerance of different Napier grass types to infection by *U. kamerunensis.* In Trial 7B, needle point injection and wound and paste methods were employed. The trials were established on a plot in Nairobi, at an altitude of 1820m.

Table 39. Inoculation methods for initiating smut infection in various hosts.

smut fungus	host	method	reference
Tilletia indica	wheat	injection of sporidia into boot using a hypodermic syringe	Warham, 1990
		high pressure spray of sporidia suspension to spike	Warham, 1990
		application of cotton wool soaked in sporidia suspension to spike	Warham, 1990
Ustilago segetum var. *nuda*		dry spores on seeds	Pandey and Gautam, 1989
Ustilago nuda	barley	injection of spores into florets using a hypodermic needle	Poehlman, 1945
		negative pressure inoculation of inflorescence	Moore, 1936
Ustilago scitaminea	sugarcane	high pressure spray of spores to buds	Bock, 1964; Byther and Steiner, 1974; Dean, 1982
		injection of spores into buds using a hypodermic syringe	Waller, 1970; Dean, 1982; Waraiich, 1989; Mohanraj *et al.*, 1987
		spore suspension applied to buds	Lloyd and Pillay, 1980
		spore paste applied to buds	Byther and Steiner, 1974
		wound and spray wound and paste	Byther and Steiner, 1974; Nasr, 1977, Waraiich, 1989
		negative pressure inoculation of canes dipping canes in spore suspension	Hirschhorn, 1949; Keshan Singh *et al.*, 1975; Nasr, 1977; Dean, 1982; Waraiich, 1989
		dipping setts	Waraiich, 1989
		dipping seeds in spore suspension	Seshadri *et al.*, 1985
		puncture bud with needle dipped in spore suspension	Ferreira, 1987

7.2 Methods

Trial 7A

Canes of Napier grass types Clone 13, Kitale bana, French Cameroon and Kakamega 1 were harvested from bulking plots maintained at KARI Muguga, in October 1997[1]. The canes were cut into lengths of three nodes each (the sheathing leaves were stripped off in case they provided mechanical protection), and kept wet overnight by immersion in a bucket of water. The following day an ustilospore suspension containing 10^6 spores per ml was made up (spore concentrations used by the workers listed in Table 39 ranged from $5x10^4$ to $5x10^6$ per ml). The 3-node canes were soaked in the ustilospore suspension for three hours, and then conditioned overnight in plastic bags at 100% relative humidity. They were then planted, in a non-sterile potting mixture (soil:sand:grit:manure in the ratios 5:2:2:1) in 20cm diameter pots, with one node above the soil surface. There were three canes per pot and ten pots per Napier grass type. Microscopic examination of the ustilospores confirmed that they were viable.

The pots were laid out as a completely randomised block and were watered as necessary. Canes were not cut back but were allowed to grow normally. Every cane was scored for the presence of disease, as indicated by the production of a smutted inflorescence. Disease presence was rated as a

---

[1] Clone 13 and French Cameroon are recognized types of Napier grass (Boonman, 1993), and Kitale bana and Kakamega 1 are local descriptions given by workers at KARI Kitale to accessions from ILRI.

simple proportion of the total number of canes that produced new stems (ie up to a maximum of 30). The trial ran for 12 months, until September 1998.

Trial 7B

Another option for the control of *U. kamerunensis* is to promote the use of a different fodder crop that is immune to infection by this pathogen. Guatemala grass (*Tripsacum laxum*) has been suggested as a possibility by forage agronomists, since it is locally available, but it was first thought necessary to confirm that this grass was not attacked by *U. kamerunensis*. Trial 7B therefore incorporated Guatemala grass. However, this grass can only be propagated by root splits and not by cuttings, and so the dipping method was not used in this trial. Instead, a modification of the needle point injection method of Ferreira (1987), and the wound and paste method (Nasr, 1977) were employed. These workers inoculated buds, but this was not possible here because Guatemala grass does not have aerial buds, and so stems were inoculated. Root splits of the same Napier grass types were tested, together with splits of Guatemala grass. Ten single stem splits of each grass type (10-20cm in length) were planted in 20cm pots, in the same potting mixture. The pots were again set out in a randomised block design and the splits were inoculated on the same day. In the needle point method, a mounted needle was dipped into an ustilospore suspension (containing 2.3×10^6 spores per ml in distilled water) and jabbed six times into the mid-line of the stem, to a depth on 0.5-1mm, just above the soil surface. The needle was flame sterilised after

every inoculation (the spore load on the needle point was less than 500 ustilospores).

In the wound and paste method, five stems of each grass type were jabbed with a flame sterilised needle, as before. The site of the punctures was then painted with a thick ustilospore suspension containing 4 x 10^8 spores per ml (obtained by crushing a smutted inflorescence in 3ml of water). There were five replicates for each treatment, and the experiment was set up in March 1998. Spore viability was confirmed, as before.

7.3 Results

In Trial 7A, the first smutted inflorescence appeared 14 weeks after inoculation, on Clone 13. Thereafter, French Cameroon and Kitale bana also showed signs of infection by *U. kamerunensis*. However, by the end of the experiment there was no sign of any infection in Kakamega 1, either from the primary attempt at inoculation, or from any secondary infection arising from ustilospores produced by the other types during the course of the trial. The results are shown graphically in Figure 27, which indicates that Clone 13 could be classified as very susceptible; French Cameroon and Kitale bana as less prone to infection, and Kakamega 1 as resistant. However, in Trial 7B, although the cuttings became well established, there was no sign of the disease after nine months.

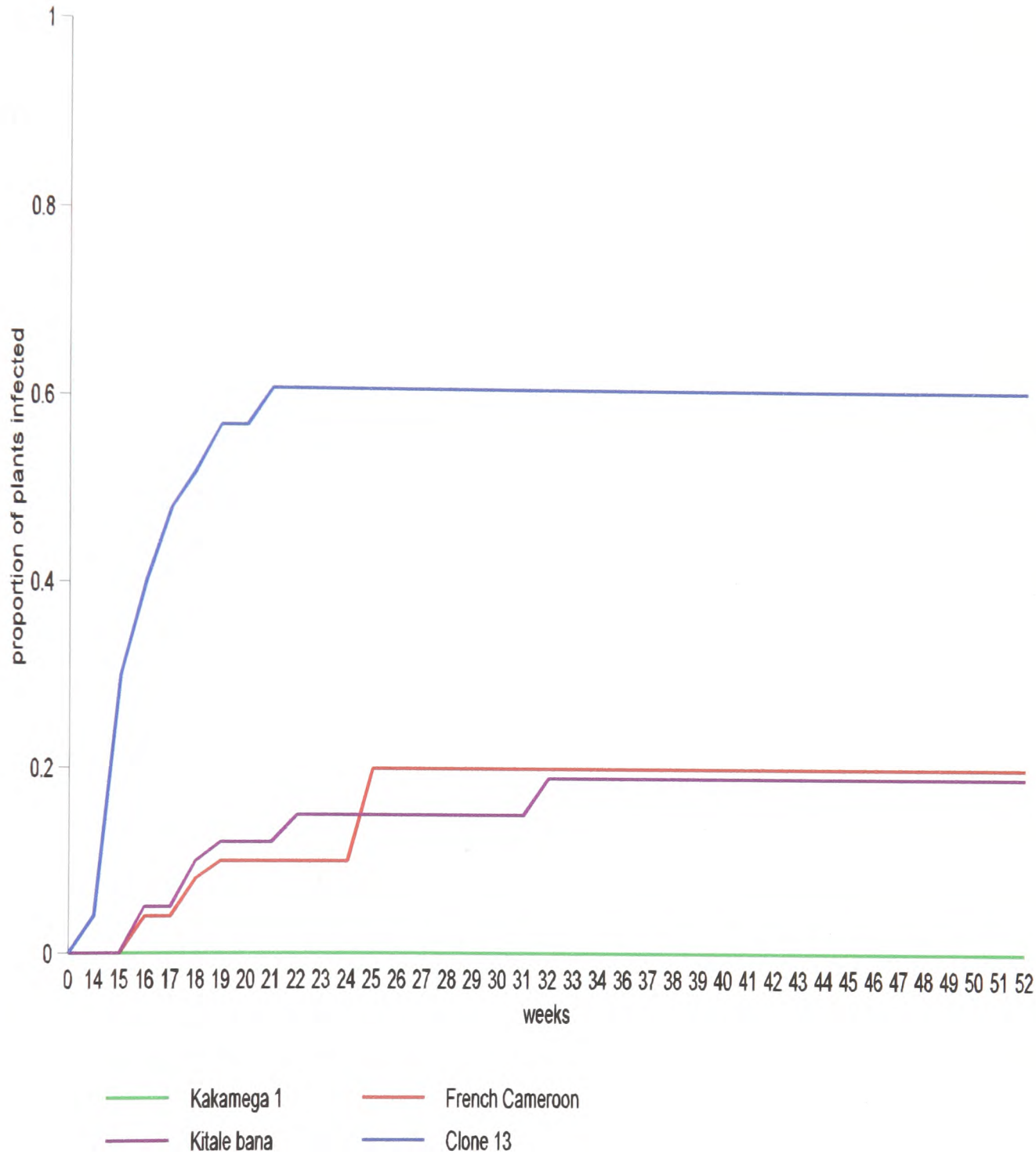


Figure 27. The proportion of stems infected by *Ustilago kamerunensis* in five types of Napier grass, using the spore dipping inoculation method.

7.4 Discussion

The results from Trial 7A suggested that resistance mechanisms occur in some Napier grass types in Kenya. It is less likely that this resistance comes from the protection offered by leaves and bud scales, as was proposed by Waller (1970) for sugarcane resistant to *U. scitaminea*, because the sheathing leaves were removed and the bud scales offered no protection when the bud elongated and emerged from their cover. However, further work on sugarcane smut has postulated the presence of chemical pre-infection resistance mechanisms (James, 1973), and glycosidic bud diffusates, that inhibit spore germination, have been identified by Rampersad and Brathwaite (1985) and Padmanaban *et al.* (1988). Similar mechanisms may play a role in the resistance of Napier grass Kakamega 1 to infection by *U. kamerunensis.*

The results from Trial 7A suggested that Kakamega 1may be immune to the smut disease and that Clone 13 was susceptible. French Cameroon and Kitale bana, with lower proportions of plants infected than Clone 13, could be exhibiting partial resistance. Partial resistance mechanisms act at different stages of the disease cycle and produce effects that reduce or delay an epidemic. Such mechanisms (usually involving the development of internal barriers) increase the incubation and latent periods and reduce sporulation (Jones, 1987), and it was noteworthy that the number of infected French Cameroon and Kitale bana plants increased more slowly than in the case of Clone 13. Increases in incubation and latency periods reduce the number of

disease cycles within a growing season, and are important in reducing the severity of epidemics in seasonal crops. However, Napier grass/smut disease is a perennial pathosystem that is not limited by changing seasons, and so partial resistance may not be effective in reducing the severity of the epidemic over the long term.

Lack of sufficient material meant that only four Napier grass types could be tested in Trial 7A, but the results demonstrated that the spore dipping method could be used to initiate infection and that differences in susceptibility and resistance were present in Kenyan Napier grass. Evaluation of additional types will be done when the bulking plots at KARI Muguga are well established.

The absence of infection in Trial 7B, in which stems were injected, supports the contention that buds are the sites susceptible to infection by the pathogen. It remains to be seen whether external resistance can be circumvented by wounding, removal of bud scales or by injection of ustilospores below the epidermis. It may also be the case that internal mechanisms of resistance are present, involving physiological conditions that are related to hereditary factors. Investigations of host protoplasm acidity, osmotic pressure and the seed germination process have been reported, but consistent correlations between these conditions and resistance to smut fungi have not been established (Fischer and Holton, 1957). There was some indirect evidence for the existence of internal mechanisms of resistance in that the Napier grass

types in Trial 7A showed variable susceptibility to infection, after the mechanical protection provided by the sheathing leaves had been removed. It is interesting to note that all infection appeared to result from the primary inoculation. There was no evidence of secondary infection, which would have been indicated by a second peak in Figure 27. This supports the contention that there is a narrow window of opportunity for infection by *U. kamerunensis*, which occurs when ustilospores come into contact with germinating buds below soil level. Plants which are not producing secondary tiller buds appear to be immune to infection.

Once inside the host, the mycelium will eventually proceed with spore formation. The time between invasion and sporulation is considered as the incubation period (Fischer and Holton, 1957). There is wide variation in the this period in the smut fungi, from as little as ten days in *U. maydis* to three years in *U. spegazzinii* (Fischer and Holton, 1957). At 14 weeks, the incubation period of *U. kamerunensis* in Napier grass is relatively short. Waller (1969) reported a six month time lapse between field infection of *U. scitaminea* and the appearance of the first smut whips, in sugarcane. It may be that a short incubation period reflects the greater ability of the pathogen to out-compete internal mechanisms of resistance offered by the host. Because the proportion of invasions that lead to production of spores and sori has not been elucidated, it is not known how successful Napier grass can be in repelling internal invasion. Based on the visual evidence of smutted

inflorescences, these fungi are assumed to be efficient pathogens. However, efficiency of the pathogen should not be accepted in the absence of evidence of the host's ability to fight off infection.

CHAPTER 8 DISEASE REMISSION

8.1 Introduction

Observations of smutted Napier grass in farmers' plots revealed that individual plants could be more or less diseased. Infected plants showed a demarcation between the smutted portion (with thin, short and sometimes fasciculated stems, thin leaves and smutted inflorescences), and the healthy part with a normal appearance. This led to the conjecture that it may be possible to remove the diseased part and free the plant of infection. If this were attainable, removal of diseased portions could be recommended as a management option that avoided the sacrifice of healthy plant material and the creation of gaps in plots (farmer' practice is to fill gaps with new cuttings, but the rate of establishment is low). It would also reduce the amount of work involved in removal of diseased plants. A trial was therefore set up to test this hypothesis. For the purpose of this experiment, a plant was defined as being in disease remission if it remained free of gross signs of disease for at least 28 weeks (it was shown in Chapter 7 that at least 14 weeks elapsed between infection and disease expression, and so 28 weeks was chosen as a reasonable length of time to be confident of disease remission).

8.2 Methods

Smut infected Napier grass plants, of different types, were collected from Kiambu District in March 1997. Every plant was separated into splits, such that each split contained some healthy and some diseased stems with roots

attached. Some splits were more heavily infected than others. Forty-four splits were planted at the spacing recommended by MoALDM (60cm between plants and 90cm between rows) in a plot at least 3km from the nearest source of natural infection. Every plant was numbered with a plastic tag and allowed to establish for two weeks. After this period all diseased stems were cut off at ground level (the first cut), leaving just the healthy portions of each plant. Thereafter, the plants were examined weekly, and if disease was observed the plants were scored for the presence or absence of *U. kamerunensis*. Smutted stems were removed on every scoring occasion, after recording their presence. The trial ran for 80 weeks, until October 1998.

8.3 Results

Eight plants showed no sign of smut infection after the first cut. This suggests that, in these stools, the smutted stems were removed completely, with no infected secondary tiller buds remaining to initiate production of a smutted inflorescence. In the 36 stools where the disease reappeared after cutting, the maximum length of time between disease appearances on the same plant was ten weeks (with a mean of 2.8 weeks $\pm$1.9, and a range of one to ten weeks). There were 33 plants in which disease remission could be identified. By the end of the trial, these stools showed no sign of disease and appeared healthy, with plentiful foliage. It can be seen from Table 40 that the longest remission period was 79 weeks and the shortest period was 50 weeks. Eleven plants died during the course of the trial, though they survived for at least ten

weeks (these stools were severely infected at the start, and it is assumed that the repeated cutting weakened them and contributed to their deaths).

Table 40. The fate of disease expression when infected portions of Napier grass are removed (1 = disease observed, 0 = disease not observed).

plant no.	weeks 1	5	11	16	20	25	28	30	35	46	65	80	remission period (weeks)
01	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
02	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
03	1 1 1 1 1 1 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	69
04	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
05	0 0 0 0 0 0 0 1 0 0 0 1 1 1 0 0 1 0 0 1 0 0 1 0 1 1							0	0	0	0		50
06	0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	76
07	0 1 1 1 1 1 0 1 1 1 0 1 1 0 0 dead												
08	0 1 1 1 1 1 1 1 0 1 1 1 1 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0							0	0	0	0	0	54
09	1 1 1 1 1 1 1 1 1 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	69
10	0 0 1 0 0 1 1 0 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0 1							0	0	0	0		50
11	0 1 0 0 0 0 1 1 1 0 1 1 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	60
12	0 1 1 1 1 0 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	69
13	1 1 1 0 0 1 1 1 0 0 0 0 1 0 0 dead												
14	0 0 0 1 0 1 1 1 0 1 1 1 0 0 0 dead												
15	0 1 1 0 0 1 0 0 1 1 0 1 0 0 0 0 0 0 0 0 1 0 0 0 1 0							dead					
16	0 0 0 0 0 0 1 1 1 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	69
17	0 0 1 0 1 0 0 0 1 1 0 1 0 1 1 0 0 1 0 0 0 0 0 1 1							0	0	0	0		50
18	0 1 1 1 0 1 0 0 0 1 0 0 1 0 0 1 0 0 0 1 0 0 dead												
19	1 1 1 1 1 1 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1							0	0	0	0		50
20	1 1 1 0 1 1 0 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 dead												
21	0 1 0 0 0 0 0 1 1 1 1 1 1 0 0 0 0 0 1 0 0 1 0 1 1							0	0	0	0		50
22	1 1 0 1 1 0 0 0 1 1 1 0 1 0 0 0 0 1 0 0 0 0 0 0							0	0	0	0		57
23	1 0 1 0 1 1 1 1 1 1 1 1 dead												
24	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
25	0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	76
26	0 1 0 1 1 1 0 1 1 1 1 1 0 0 0 dead												
27	0 0 0 0 1 1 0 1 0 1 1 1 1 0 0 1 1 0 0 1 0 0 1 0 0							0	0	0	0	0	53
28	1 1 0 1 1 1 0 1 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	69
29	0 1 1 1 1 0 0 0 0 0 1 dead												
30	1 1 1 0 1 1 1 1 1 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0							0	0	0	0	0	57
31	0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	77
32	0 0 0 1 0 0 0 1 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0							0	0	0	0	0	54
33	0 1 1 1 1 0 1 1 1 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0							0	0	0	0		57
34	0 0 0 1 0 1 1 1 1 1 1 1 0 0 0 1 0 0 1 0 0 0 0 0							1	0	0	0	0	50
35	0 1 0 1 1 0 0 0 0 1 0 1 1 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	64
36	0 0 1 0 1 0 0 1 1 1 1 1 1 0 0 1 0 0 1 0 0 1 0 0							1	0	0	0	0	50
37	0 0 1 1 0 0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0							1	0	0	0	0	50
38	1 0 0 0 1 1 1 1 1 1 1 1 0 0 0 1 0 0 1 0 0 0 0 0							0	0	0	0	0	57
39	0 0 0 1 0 0 1 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0							0	0	0	0	0	57
40	1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 0 dead												
41	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
42	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
43	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							0	0	0	0	0	79
44	0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 0 dead												

8.4 Discussion

In the case of systemic infection in a perennial host, the mycelium is also perennial and usually produces a crop of spores continuously, as long as the host survives (Fischer and Holton, 1957). This is the situation found in Napier grass infected by *U. kamerunensis.* Once a stem has become infected, the fungus continues to induce the production of smutted inflorescences, until the stem is so weakened that it dies. In the cereal smuts, the mycelium is thought to keep pace with the growing point of the host (Fischer and Holton, 1957), although Vanderwalle (1942) has observed that in loose smut of wheat the fungus lags behind development of the host and eventually catches up near the time of floral differentiation. The fact that *U. kamerunensis* can initiate production of a smutted head in as little as 14 weeks after inoculation, and that it induces early flowering, suggests that this fungus keeps pace with the growing point in Napier grass. Although the presence of mycelium was demonstrated within infected stems, and the pathogen travels up the stem as it grows, there is no evidence that other parts of the stool can be infected from the original invasion. This was borne out by observations of diseased plants in the field in which healthy and infected stems can be seen on the same stool. It appears, therefore, that the fungus cannot move within a stem against the direction of plant growth, to infect other stems on the same stool. The pathogen can be described as vertically systemic but not horizontally systemic. Therefore, in stools with more than one infected stem, every stem must have been invaded individually. The localisation of the fungus within infected stems

means that disease remission can be obtained in the rest of the stool if the diseased stems are removed, as was demonstrated in this trial. It would be useful to assess remission in different Napier grass types and across the disease classes developed in Chapter 6, to determine the overall effect on the plant.

Disease remission has been described in pearl millet (*Pennisetum americanum*), infected by the downy mildew pathogen, *Sclerospora graminicola* in India, Mali and Niger (Singh, 1988). Singh (1989) reported a similar occurrence in sorghum attacked by the downy mildew *Peronosclerospora sorghi* in Zimbabwe. He referred to the development of symptomless shoots from systemically infected plants as disease recovery. The phenomenon of disease remission may be common in tussock grasses (J M Lenné, *pers. comm.*), and merits further investigation.

It was also apparent that buds above the site of the original invasion will become colonised by the fungus and will produce smutted heads, if the buds germinate. A similar occurrence has been described for sugarcane smut, in which all canes produced from an infected primary bud were diseased; an infected secondary tiller bud gave rise to disease only in that tiller (Waller, 1970).

Because the mycelium of *U. kamerunensis* remains localised it is possible to recommend removal of diseased stems as a management technique, and this has been adopted by farmers in Kiambu District where the disease is particularly serious. The trial described here was a severe test for the plants since they were subjected to stress from transplantation, as well as from fungal attack. Napier grass plants in farmers' fields should have better recovery rates since they will not be exposed to the same transplantation stress.

CHAPTER 9 CONCLUSIONS

The demand for information on smut disease of Napier grass originated from small-holder dairy farmers in Kiambu District, and it was in this context that the investigations reported in this thesis were carried out. Historically, very little work had been done on fodder crops in Kenya, and so the outbreak of smut disease of Napier grass caught Kenyan small-holder dairy farmers unprepared. Suddenly they were faced with a new threat to the major source of fodder for their zero grazed cattle. Many farmers did not even recognise that the decline in vigour and biomass of the crop was caused by a disease; rather it was regarded as just a different type of plant growth. This misunderstanding may have contributed to the delay in notification of the disease to the local extension services and the national programme. Even when the condition was properly identified, there was little advice that could be offered for its management, beyond cutting and burning of affected stools. There was no information on the biology, ecology or epidemiology of the pathogen, and farmers had no indigenous technical knowledge to fall back on as the disease was unknown to them.

The starting point, when faced with a pathogen on which no work had been reported since its first description at the beginning of the 20th century, and with a crop whose pathology was in all respects poorly understood, was to elucidate the basic biology and ecology of the pathogen. Results from these

investigations revealed a smut fungus typical of the Ustilaginaceae in terms of morphology and habit. The main value of this part of the work was in suggesting some avenues of investigation for the development of management advice, particularly the search for host plant resistance and options for making use of the phenomenon of disease remission.

Reasons for the increase in incidence of *Ustilago kamerunensis* and its severity in Kenya were suggested in Chapter 2. There has been a documented increase in the use of, and area given over to, Napier grass cultivation (Staal *et al.*, 1997), as a result of pressure on communal pastures and the promotion of zero grazing by NGOs and government agencies (Boonman, 1993; Valk, 1990). It is not known whether the severity of the disease in Kenya can be explained by the emergence of a more virulent strain of *U. kamerunensis* or by the fact that the types of Napier grass in the country are more susceptible than those found in other countries where the disease has not been reported as serious. However, the expansion of the disease in Kenya can probably be attributed to the enlargement in the area of Napier grass under cultivation, rather than to an increase in virulence of the fungus.

No pathogenicity tests were done, and the existence of *U. kamerunensis* biotypes was not demonstrated. It is possible that physiologic races of the pathogen have not evolved because the types of Napier grass that the fungus attacks are very similar genetically (Lowe *et al.,* in preparation), hence obviating

the need for sexual recombination by the pathogen. However, races have been described in many other *Ustilago* species, such as *U. avenae*, *U. maydis*, *U. violacea, U. nuda, U. hordei* and *U. scitaminea* (Fischer and Holton, 1957; Comstock and Heinz, 1977), suggesting that development of physiologic specialisation is beneficial, in terms of pathogenicity, to the smut fungi. It would not be surprising if investigations of the pathogenicity of *U. kamerunensis* also demonstrated the existence of races in this species.

Results of the pest and disease survey suggested that *U. kamerunensis* poses the major pathological threat to Napier grass in Kenya. Other diseases and insect pests were minor in incidence and severity and their presence was reported in earlier pest surveys (Nattrass, 1961) (Chapters 2 and 3). Prior to the present survey, it was thought likely that smut disease would eventually be found in all areas of Napier grass cultivation. However, the model of smut proportion described in Chapter 3 identified areas at risk of the disease as being between 1800 and 2400 masl. This is valuable information in that it allows management advice to be targeted at those areas most at risk from the pathogen, thus maximising the use of limited resources. In addition (though recognising that restrictions in the movement of crops by quarantine regulations within a country is very difficult to enforce), it is at least now possible to identify high and low risk locations for farmers and government agricultural officers, so that movement of infected planting material between such areas can be minimised.

The model of *U. kamerunensis* probability derived here was of the predictive type, designed to help in understanding of the disease system and the factors that influenced it. The only factor that explained the probability of Napier grass smut was based on a quadratic function of altitude. The altitude function may well be composed of sub-factors, such as soil fertility or rainfall. Evidence presented in Chapter 5 suggested that temperature is unlikely to be a component of the altitude effect. The area under the curve can be divided into three altitude groups ie *smut absent* (below 1880 and above 2400 masl), *smut absent and present* (1800-2000 and 2300-2400 masl) and *smut present* (2000 - 2300 masl). This may of course reflect too small a sample size (it is accepted that a record of no disease in the *smut absent* range may simply imply that the plot has not been exposed to inoculum), or there may be some biological meaning, as yet unquantified. Nonetheless, it is believed that this model is the first of its kind to offer a means of identifying geographical locations, likely to be at risk from a smut disease.

Evidence was provided in Chapter 5 for the relatively short duration of ustilospore viability in *U. kamerunensis.* Although the soil moisture content was higher than normal during Trial 5A, because of heavy rain, the spore longevity period was not much increased under drier soil conditions. Even spores stored in a desiccator, at very low relative humidity, lost their ability to germinate after 14 weeks. It is possible that the short longevity period of *U. kamerunensis* may provide an opportunity for management of the disease using

fallow periods, if diseased plants are removed and there are no others in the vicinity. In addition, the death of ustilospores at a temperature of 40°C suggests that hot water treatment of cane pieces could be recommended. This would kill spores on the outside of the canes but would probably have no effect on mycelium already growing systemically within. Even so, the development of a method for hot water treatment may be worthy of consideration.

Evidence was also presented in Chapter 5 that, in common with other smut fungi which infect their hosts in the soil, the infection court for *U. kamerunensis* is the germinating tiller bud. Infection cannot take place unless ustilospores reach the inner meristematic regions of Napier grass buds. The protection afforded by bud scales and sheathing leaves (whether physical and/or physiological) is lost when buds grow, thus exposing meristems to the infection process. In grasses, tiller buds may arise just above or below the soil surface (Chapman, 1996), but it appears that only those below soil level are at risk from the Napier grass pathogen, despite accumulations of spores in aerial tiller buds.

Despite concerted efforts (described in Chapter 5) to establish an epidemic of *U. kamerunensis* in a trial plot of Napier grass, over a period of 18 months, no epidemic resulted. Campbell and Madden (1990) quote Gäumann as listing three main conditions that must occur simultaneously before an epidemic can develop. These conditions are an abundant supply of susceptible individuals,

the presence of an aggressive pathogen and optimal weather conditions for the development of the pathogen. Evidence presented here suggests that the first and last conditions were met, but that *U. kamerunensis* does not have a high epidemic potential, being limited by the narrow window of opportunity (growing tiller buds) available for initiation of infection.

The description in Chapter 6 of a method for measuring disease magnitude is the first example of a severity scale devised for a smut disease. There were some specific attributes of the Napier grass/*U. kamerunensis* pathosystem that allowed the development of the scale, particularly the discrete growth habit of Napier grass, the presence of a perennial pathogen in a perennial host and the vertically systemic nature of the fungus within the host. These factors limit the wider application of the severity scale and the field assessment method. However, a similar scoring method could be produced for the sugarcane smut system, which exhibits many features in common with smut disease of Napier grass.

It could be argued that the losses caused by the *U. kamerunensis* are so variable that it is not worthwhile assessing biomass reduction in the first place. For example, it was shown in Chapter 6 that losses typically range from 25 to 46 percent, if healthy parts of diseased stools are saved, or 51 to 89 percent if the whole stool is sacrificed. The uncertainty is compounded by the difficulty in relating these figures to reductions in milk production and farm incomes.

However, there remains the need to quantify the effects of *U.kamerunensis* on Napier grass to justify interventions, in competition with demands for control of other diseases on other crops. Calls from farmers for assistance also deserve to be met, and support for them depends on convincing funding agencies to provide inputs for the development of management techniques. The first concern of funders is the economic benefit to be gained from their input, and this requires that some assessment of loss is made. Allied to this is the difficulty of estimating the extent of loss from the inspection of a diseased plot. Some farmers reported smut infections as severe. Others were surprised during the survey to be told that they had any disease in their Napier grass plot. In neither case was there any appreciation of the real impact of the pathogen. Disease severity is usually overestimated during inspection of a stand (Kranz, 1988), but in the case of *U. kamerunensis* it became apparent that the effects of this pathogen tended to be underestimated. Many disease systems are non-intuitive, and the Napier grass/*U.kamerunensis* interaction is such a system.

Testing for resistance to smut disease revealed one Napier grass type (Kakamega 1) that showed no signs of the disease, in comparison with susceptible types that developed symptoms 14 weeks after inoculation (Chapter 7). Kakamega 1 has been released to farmers for bulking and on-farm evaluation, in comparison with bana, which is currently the favoured type.

The sheathing leaves were removed in this trial, and so it is postulated that some internal mechanism of resistance is operating in Kakamega 1, possibly involving physiologic conditions or the formation of internal barriers or lignification of host tissue that limits the development of fungal hyphae or haustoria.

The use of resistant varieties is likely to offer the best option for management of the disease in the long term, for this traditionally low-input crop. However, Kenyan Napier grass types come from a very narrow genetic base, and so any resistance may not be stable in the face of a continuous challenge from the pathogen. Enlarging the proportion of resistant types within the Kenyan population of Napier grass will increase selection pressure on the fungus to evolve mechanisms to overcome it. A breeding programme is not recommended because information is lacking on the type of resistance (active or passive), its genetic control (oligogenic or polygenic) and the genetic variability of the pathogen. In the short term, further testing of indigenous Napier grass is best done on-farm by distributing identified types so that farmers can manage the testing process themselves, but this needs to be well coordinated to prevent the spread of diseased material. The ultimate test of success is the production of disease-resistant plants, and this can be more quickly demonstrated on farmers' plots, given the nature of normal farmer-to-farmer dissemination of planting material. Assessment on farmer's plots would speed up the selection process, rather than reliance on traditional testing on-station which is very time

consuming.

The main difficulty in field work with Napier grass lies in distinguishing different types. They are morphologically very similar, and it takes a great deal of practice to be confident of correct identification. It was for this reason that Napier grass type was not included in the development of the model of smut probability, because the farmers were unable to identify the types in their plots. During recent participatory rural appraisals carried out in Kiambu and Thika districts, farmers claimed that varieties they called Panama and Denmark were resistant to smut disease (Musembi and Nyanyu, 1998). However, these are not accepted names for any known variety, and are thought to result from the activities of a Danish NGO working in the area. They were in fact identified as Bana (G Karanja, *pers. comm.*). This highlights the need for a simple field guide to identification of Napier grass types, for farmers, extensionists and researchers.

With regard to artificial inoculation, dipping canes in ustilospore suspension induced infection in the susceptible types, but the more invasive methods of spore pasting, wounding and needle inoculation did not. This confirms the belief that the fungus can only gain entry through undifferentiated tissues, as found in actively growing meristems. It appears unlikely that the infection process can be "short circuited" by direct injection of spores into stems or dormant buds.

The case for disease remission was argued in Chapter 8. The presence of smut disease in a Napier grass plant is made visually obvious by the transformation of the inflorescence into a mass of black spores. This makes the task of identification easier for farmers and facilitates the removal of smutted stems. Cutting and burning of such stems is straightforward and involves little extra labour, though regular inspection of the plot is needed. If diseased stems are removed as soon as they appear the risk of reinfection is greatly reduced, as was demonstrated in this chapter. Napier grass plants remained disease free (after removal of smutted stems) for up to a year, despite the continuous presence of nearby disease inoculum. In the course of many visits to farms it was discovered that some farmers were already removing the diseased parts of Napier grass stools, the beginnings, perhaps, of indigenous technical knowledge in the management of smut disease of Napier grass.

There is no evidence of alternative hosts for *U. kamerunensis*, either wild or cultivated (the only other confirmed smuts on *Pennisetum* spp. in Kenya are *U. scheffleri* on *P. clandestinum* and *Tolyposporium penicillarie* on *P. typhoides*). This simplifies control of *U. kamerunensis* and aids the eradication of limited outbreaks through the removal of diseased Napier grass plants. However, there are examples of *Ustilago* species that infect non-host plants in other countries, sometimes in different genera, such as *U. scitaminea* in Nigeria that attacks the sedge *Cyperus dilatatus* (Olufolaji, 1987). *Cyperus* species are common weeds in Kenya (Terry, 1984), particularly at the higher altitudes above 1000m that

also favour the cultivation of Napier grass. The increase in incidence of *U. kamerunensis* makes it more likely that other plant species will be exposed to infection pressure from the fungus, such that alternative hosts may develop and become important sources of inoculum in the future.

It is not known why Napier grass smut disease first appeared in Kenya near Nairobi. If the source of the inoculum was wind-borne ustilospores from Tanzania or Uganda, why were Napier grass areas close to these borders not primarily infected? A possible explanation is that the first outbreak resulted from the import of diseased material. There is continuous movement of planting material of many crops along the road between the lake region and the Kenyan coast, and this may be the original route of *U. kamerunensis* into the country. Further spread of the pathogen is likely, limited perhaps to higher altitudes, through dissemination of wind-borne ustilospores and diseased canes. The continuous cropping pattern of Napier grass will also assist the epidemic, since there is no fallow period to break the cycle of infection.

It was suggested above that resistant varieties offer the best hope for the long term, affordable, control of *U. kamerunensis*. Nonetheless, the development of other control options may be warranted. The pathogen has a very narrow window of opportunity to begin infection, namely when tiller buds are growing through the soil, and it may be possible to control the disease at this stage by dipping canes in fungicide prior to planting. Systemic compounds would be

required to simultaneously destroy latent infection within the cane. Triconazoles (ergosterol biosysnthesis inhibitors - EBIs) may be suitable, since these are recommended as seed dressings to control cereal smuts (Tomlin, 1997). EBIs have been used successfully to manage *U. bullata* on *Bromus willdenowii* in New Zealand (Falloon and Rolston, 1986). Two triconazole compounds are licensed for use in Kenya (PCPB, 1997).

There are some bio-control interventions that could be explored, targeting the external forms of the fungus (it is difficult to see how a bio-control agent could gain access to the pathogen once it has entered the host). The spore mass may be susceptible to attack by predators or antagonists. Fischer and Holton (1957) give several examples of fungi (in the genera *Fusarium, Alternaria* (=*Macrosporium*), *Oospora* (=*Oidium*), *Trichothecium* and *Acontium*) that parasitize *Ustilago* spp. Fischer and Holton (1957) also reported instances of bacterial antibiosis against *Ustilago, Sphacelotheca* and *Tilletia* spp. Some of these bacteria have been field tested and shown to inhibit smut spore viability (Ragab, 1994). Antagonistic fungi have also been identified in smuts of oat, wheat, barley and millet (Fedoseva *et al.*, 1979), and sugarcane (Vaishnav *et al.*, 1992; Sinha and Kishan Singh, 1983).

Pruett and Colque-A (1984) presented evidence that some beetle species were controlling smut disease of sugarcane in Bolivia, by feeding on the spore mass. However, in Cuba, other Coleoptera have been implicated as vectors of the

disease (Acevedo *et al.*, 1984), and so the use of insects as bio-control agents may not be viable.

Farmers could be persuaded to try alternative fodder crops such as *Calliandra*, legumes, Guatemala grass or giant setaria, using demonstration plots on their farms. However, anecdotal evidence suggests that the adoption of new crops is unlikely, unless the smut disease of Napier grass becomes more severe and there are no other management options available.

The epidemic of *U. kamerunensis* is in its early stages and now is the best time for its management, using the simple and affordable technique of roguing diseased plants. Nonetheless, further spread of the disease cannot be ruled out, and should this occur, sustainable management may require the deployment of additional control options such as the introduction of resistant varieties and appropriate bio-control agents.

The discoveries outlined in Chapters 7 and 8 were incorporated into an advisory leaflet for farmers and extension workers, which is given in Appendix 7 (Farrell, 1998). Actions to be taken included frequent crop inspection leading to removal and burning of smutted plants, using disease-free planting material, improving the health of the stand by fertilizer or manure applications and suggestions for the replacement of Napier grass by other fodder grass species. It is not claimed that this list is exhaustive, and issuing the bulletin early in the

investigation may be thought premature. However, it was distributed to satisfy repeated demands from farmers for advice on how to contain a new threat to Napier grass, that they thought posed a serious challenge to the well being of their cattle. Some of the advice has already been modified. For example, it is now suggested that only the diseased portions of the plant need be removed (not the whole stool), and that proposing the use of other fodder grasses is unlikely to be of value, given that there is a great reluctance on the part of farmers to cultivate alternatives that are much slower growing than Napier grass, and not as palatable. The aim of the bulletin was to provide farmers with a basket of options, from which farmers could draw as they saw fit, depending on their needs and resources, rather than offering an integrated package.

Kiambu, the area of the present study, is the third most densely populated district of Kenya, with over 200,000 households and about 350 people per km^2 (CBS, 1996). Pressure for land is intense, with very little land not under cultivation. Even roadside verges are given over to growing crops, usually Napier grass, despite government regulations that forbid this practice (Mutisya and Lado, 1991). In such a farming system, where the farm size is small and land is continually subdivided through inheritance, dairy cattle may be the most productive agricultural method for income generation. Now, and for the foreseeable future, these zero grazed cattle will rely on the provision of a constant supply of healthy Napier grass.

References

Acevedo R, Lopez E, Fuentes A and Gonzalez R (1984) Biological results on *Cercyon* sp. (Coleoptera: Hydrophilidae), an insect associated with sugarcane smut. *Centro Agricola* **11,3**:112.

Adeoti A A and Adeniji M O (1982) Occurrence of narrow brown leaf spot disease of rice in Nigeria. *Oryza* **19,1**:56-57.

Ainsworth G C (1973) Introduction and keys to higher taxa. In: G C Ainsworth, F K Sparrow and A S Sussman (eds) *The fungi - an advanced treatise, Vol. 4A.* Academic Press, New York. pp. 1-7.

Alexander A G (1985) *The Energy Cane Alternative*. Elsevier, Amsterdam, The Netherlands. pp. 377-414.

Alexander H M, Thrall P H, Antonovics J, Jarosz A M and Oudemans P V (1996) Population dynamics and genetics of plant disease - a case study of anther smut disease. *Ecology* **77(4)**:990-996.

Alexopoulos C J and Mims C W (1979) *Introductory Mycology, 3rd Edition.* Wiley, New York. pp. 632.

Alghali A M and Domingo J S (1982) Weed hosts of some rice pests in North Western Sierra Leone. *International Rice Research Newsletter* **7,2**:10.

Amorim L, Bergamin Filho A and Hau B (1993) Analysis of progress curves of sugarcane smut on different cultivars using functions of double sigmoid pattern. *Phytopathology* **83,9**:933-936.

Anderson J B, Kohn L M and Leslie J F (1992) Genetic mechanisms in fungal adaptation. In: G C Carroll and D T Wicklow (eds) *The Fungal Community: its Organization and Role in the Ecosystem*. Marcel Dekker Inc., New York. pp. 73-98.

Andreis H J (1980) Viability of sugarcane smut spores in a Florida organic soil at three moisture levels. *The Sugar Journal* **May 1980**:21-22.

Anon. (unpublished (a)) *Index to the Fungi of Kenya*. Plant Pathology Section, National Agricultural Research Laboratories, Nairobi, Kenya.

Anon. (unpublished (b)) *Kenya List of Fungi, Parts 1 and 2.* Plant Pathology Section, National Agricultural Research Laboratories, Nairobi, Kenya.

Anon. (1947) The measurement of potato blight. *Transactions of the British*

Mycological Society **31**:140-141.

Anon. (1952) Department of Agriculture Annual Report. Government Printer, Nairobi, Kenya. pp. 239.

Anon. (1974a) Annual Report for the year 1974. Ministry of Agriculture and Natural Resources, Mauritius. Report No. 2. pp. 139.

Anon. (1974b) Annual Report 1974. Central Plantation Crops Research Institute, Kasaragod, India. *Plant Nematology*. pp. 61-63.

Anon. (1977) Taiwan Sugar Research Institute, Annual Report 1976-1977. pp. 65.

Anon. (1985) Central Plantation Crops Research Institute, Annual Report 1984.

Anon. (1998) In: *Proceedings of the 2nd Napier Grass Workshop*. KARI/DFID NARP II, Nairobi, Kenya. pp. 46.

Antonovics J (1998) The population biology of infectious disease: perspectives for plant pathology. In: *Proceedings of the 7th International Congress of Plant Pathology, 9-16 August 1998, Edinburgh, Scotland.*

APS (1997) Archived Transcript of the APS Karnal Bunt Symposium, 24 June - 16 August 1996. *APSNet* [http://www.scisoc.org/+resourc/karnal] 1997.

APS (1998) Introduction to Karnal bunt. *Karnal Bunt Symposium. American Phytopathological Society* [http://www.scisoc.org/resource/karnal/kbspaper/artintro.htm] 1998.

Bahat A (1985) Glufosinate ammonium for general weed control in vineyards, citrus and other fruit orchards, and uncultivated areas. *Phytoparasitica* **13,3/4**:239.

Bauer R, Oberwinkler F and Vánky K (1997) Ultrastructural markers and systematics in smut fungi and allied taxa. *Canadian Journal of Botany* **75,8**:1273-1314.

Bell F H (1951) Distribution of hyphae of several plant pathogenic fungi in leaf tissue. *Phytopathology* **41**:3.

Bindra O S and Singh J (1971) Biological observations on the white grub, *Lachnosterna* (*Holotrichia*) *consanguinea* Blanchard

(Coleoptera:Scarabaeidae) and the relative efficiency of different insecticides against the adults. *Indian Journal of Entomology* **33,2**:225-227.

Bisby G R and Ainsworth G C (1943) The numbers of fungi. *Brit. Myc. Soc. Trans.* **26**:16-19.

Bock K R (1964) Studies on sugar cane smut (*Ustilago scitaminea*) in Kenya. *Transactions of the British Mycological Society* **47**:403-417.

Bogdan A V (1955) Herbage plants in at the Grasslands Research Station, Kitale, Kenya. *East African Agricultural and Forestry Journal* **20**:151-165.

Bogdan A V (1977) *Tropical Pasture and Fodder Plants*. Longman, UK. pp. 475.

Bolker M and Kahmann R (1993) Sexual pheromones and mating response in fungi. *Plant Cell* **10**:1461-1469.

Boonman J G (1993) *East Africa's Grasses and Fodders: Their Ecology and Husbandry.* Kluwer Scientific Publications, The Netherlands. pp. 343.

Bowden J (1976) Stem borer ecology and strategy for control. *Annals of Applied Biology* **84**:107-111.

Brefeld O (1895) *Untersuchungen aus dem Gesammtgebiete der Mykologie XII: Hemibasidii Die Brandpilze III*. Münster, Germany. pp. 137.

Brunken J N (1977) A systematic study of *Pennisetum* sect. *Pennisetum* (Graminae). *American Journal of Botany* **64(2)**:161-176.

Brunt A, Crabtree K and Gibbs A (1990) *Viruses of Tropical Plants*. CAB International/ACIAR. pp. 707.

Burton G W (1989) Registration of Merkeron Napier grass. *Crop Science* **29,5**:1327.

Byther R S and Steiner E W (1974) Comparison of inoculation techniques for smut disease testing in Hawaii. In: *Proceedings of the XVth Congress of the International Society of Sugarcane Technologists.* pp. 280-288.

Campbell C L and Madden L V (1990) *Introduction to Plant Disease Epidemiology.* John Wiley and Sons, New York, USA. pp. 532.

CBS (1989) *Statistical Abstract.* Central Bureau of Statistics, Ministry of

Planning and National Development. Nairobi, Kenya. pp. 276.

CBS (1993) *Pre-Population Census Coverage of Households by Sub-Location.* Central Bureau of Statistics, Ministry of Planning and National Development. Nairobi, Kenya.

CBS (1996) *Statistical Abstract.* Central Bureau of Statistics, Ministry of Planning and National Development. Nairobi, Kenya. pp. 325.

Chapman G P (1996) *The Biology of Grasses*. CAB International, Wallingford, UK. pp. 273.

Chatterjee S N and Nimbalkar M R (1977) Occurrence of maize stripe disease in Maharashtra. *Indian Journal of Agricultural Sciences* **48,9**:427-429.

Chavangi N A (1987) General role of women in the dairy industry in Kenya. *Bulletin of the International Dairy Federation* **221**:130-134.

Chiarrappa L (ed) (1971) *Crop Loss Assessment Methods, Supplement 1.* FAO Rome and Commonwealth Agricultural Bureaux, UK.

Chikafumbwa F J K T, Costa Pierce B A, Jamu D M, Kadongola W K and Balarin J D (1993) Investigations on the use of on-farm resources as pond inputs to culture *Tilapia rendalli* and *Ochreochromis shiranus* on smallholder farms in rural Malawi. *Aquaculture* **117,3-4**:261-271.

Ciferri R (1953) A subgeneric division of the genus *Ustilago* Rouss. ex Pers. *Atti. Ist. Bot. Univ. Pavia* **Ser. 5,10**:99-104.

Ciferri R (1963) The Glomosporiaceae family (Ustilaginales) *Riv. Pat. Veg. Pavia* **Ser. 3,3**:141-143.

Comstock J C and Heinz D J (1977) A new race of culmicolous smut of sugarcane in Hawaii. *Sugarcane Pathologist's Newsletter* **19**, 24-25.

Cox D E (1976) A new homobasidiomycete with anomalous basidia. *Mycologia* **68**:481-510.

Cox D R and Snell R J (1989) *The Analysis of Binary Data.* 2nd Edition. Chapman and Hall, London, UK.

Cunfer B M (1993) Diseases of rye (*Secale cereale* L.). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/rye] 1998.

Cunningham G H (1924) The Ustilaginales , or "Smuts", of New Zealand. *Trans. and Proc. New Zealand Inst.* **55**:397-433.

Dawar K K and Singh V P (1975) The influence of smut on the productivity of *Iseilema laxum* Hack. *Current Science (India)* **44 (23)**:866-867.

Dean J L (1982) The effect of wounding and high-pressure spray inoculation on the smut reactions of sugarcane clones. *Phytopathology* **72,8**:1023-1025.

Deeming J C (1982) Hostplant records for some Nigerian *Diopsis* species (Diptera:Diopsidae). *Entomologist's Monthly Magazine* **118**:1420-1423.

Dennis R W G (1988) *Melanotaenium majus*, an apparently overlooked African smut. *Trans. Brit. Myc. Soc.* **90**:471-472.

Duke M M (1926) Fungi from Kenya colony. *Kew Bulletin* **8**:305.

Duran R (1973) Ustilaginales. In: G C Ainsworth, F K Sparrow and A S Sussman (eds) *The fungi - an advanced treatise*. Vol. 4B. Academic Press, New York. pp. 281-300.

Duran R and Safeeulla K M (1968) Aspects of teliospore germination in some North American smut fungi. *Mycologia* **60**:231-243.

Ellis S and Mellor A (1995) *Soils and Environment*. Routledge, London, UK. pp. 364.

Ellison C A and Evans H C (1990) Preliminary studies to assess the potential of fungal pathogens as biological control agents for *Rotboellia cochinensis* (Graminae). In: E S Delfosse (ed) *Proceedings of the VII International Symposium on Biological Control of Weeds*. Ministero dell'Agricoltura e delle Foreste. Rome/CSIRO. pp. 477-482.

Elston D A and Simmonds N W (1988) Models of sugarcane smut disease and their implications for variety testing. *Journal of Applied Ecology* **25**:319-329.

Emmett RW and Parbery D G (1975) Appressoria. *Annual Review of Phytopathology* **13**:147-167.

Epstein A H and Simons M D (1993) Diseases of oats (*Avena sativa* L). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/oats] 1998.

Falloon R E and Hume D E (1988) Productivity and persistence of prairie grass (*Bromus willdenowii* Kunth). 1. Effects of the head smut fungus *Ustilago bullata* Berk. *Grass and Forage Science* **43**:179-184.

Falloon R E and Rolston M P (1986) Head smut of prairie grass controlled by treating seed with ergosterol biosynthesis inhibitors. In: *Proceedings of the 39th New Zealand Weed and Pest Control Conference.* New Zealand Weed and Pest Control Society. Palmerston North, New Zealand. pp. 54-59.

Falloon R E, Rolston M P and Hume D E (1988) Head smut of prairie grass and mountain brome: a review. *New Zealand Journal of Agricultural Research* **31(4)**:459-466.

Farrell G (1998) A new disease of Napier grass: management advice for farmers. KARI/DFID NARP II Information Leaflet. 2pp.

Fang Y S (1994) Identification of *Xiphinema* spp. (Nematoda: Dorylaimoidea) around the roots of *Pennisetum purpureum* in a suburb of Guangzhou. *Journal of Yunan Agricultural University* **9,1**:7-10.

FAO (1978) Report on the Agro-ecological Zones Project. Methodology and Results for Africa. FAO, Rome, Italy.

FAO-Unesco (1974) *Soil Map of the World: Volume 1, Legend.* Unesco, Paris, France.

Fedoseeva Z N, Kharchenko S N, Grebenchuk E A, Zubko I Ya and Sobolevskaya A I (1979) Penicillium species in the control of smut and powdery mildew of cereals. *Sel'skokhozyaistvennaya Biologiya* **14,2**:220-223.

Ferreira S A (1987) A new sugarcane smut inoculation method. *Phytopathology* **77,12**:1750.

Ferreira S A and Comstock J C (1993) Diseases of sugarcane (*Saccharum* spp. hybrids). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/sugarcane] 1998.

Fischer G W (1936) The longevity of smut spores in herbarium specimens. *Phytopathology* **26**:1118-1127.

Fischer G W (1945) The mode of infection and the incubation period in the stem smut of grasses, *Ustilago spegazzini* (*U. hypodytes*). *Phytopathology* **35**:525-532.

Fischer G W (1953) *Manual of the North American Smut Fungi*. Ronald Press, New York. pp. 343.

Fischer G W and Holton C S (1957) *Biology and Control of the Smut Fungi*. Ronald Press, New York. pp. 622.

Fullerton R F A and Langdon R F N (1969) A study of some smuts of *Echinochloa* spp. *Proceedings of the Linnean Society of New South Wales.* **93**:281-293.

FURP (1987) *Description of the First Priority Sites in the Various Districts.* Ministry of Agriculture Fertilizer Use Recommendation Project, Final Report, Annex II, Kiambu District. Vol. 20. pp. 37.

Gahukar R T (1990) Population ecology of *Acigona ignefusalis* (Lepidoptera:Pyralidae) in Senegal. *Environmental Entomology* **19,3**:558-564.

Gatumbi R W (1985) Host lists of Kenya fungi and bacteria (a ten year supplement 1971 - 1980). *Special Issue of the East African Agricultural and Forestry Journal.* pp. 15.

Gaumann E (1946) *Pflanzliche Infektionslehre*. Birkhäuser, Basel, Switzerland. pp. 611.

Gebre-Amlak A and Amlak A-Gebre (1988) Development of maize stalk borer, *Busseola fusca* (Fuller), in wild host plants in Ethiopia. *Journal of Applied Entomology* **106,4**:390-395.

Gibbons J D (1976) *Non-parametric Methods for Quantitative Analysis*. Holt, Rinehart and Winton, New York, USA. pp. 463.

Glaz B, Ulloa M F and Parroda R (1989) Yield effects of sugarcane smut infection in Florida. *Journal of the American Society of Sugar Cane Technologists.* **9**:71-80.

Goates B J (1998) Epidemiology of Karnal bunt of wheat. *Karnal Bunt Symposium*. *American Phytopathological Society* [http://www.scisoc.org/resource/karnal/kbspaper/artepid.htm] 1998.

Godfrey G H (1935) Hitherto unreported hosts of root-knot nematode. *The Plant Disease Reporter* **XIX,4**:29-31.

Govinthasamy T and Cavers P B (1995) The effects of smut (*Ustilago destruens*) on seed production, dormancy and viability in fall panicum

(*Panicum dichotomiflorum*). *Canadian Journal of Botany* **73,10**:1628-1634.
Gregory P H and Stedman O J (1953) Deposition of airborne *Lycoperdium* spores on plane surfaces. *Annals of Applied Biology* **40**:651-674.

Häfliger E and Scholz H (1980) *Grass Weeds 1*. CIBA-GEIGY, Basle, Switzerland. pp. 142.

Harada J, Zungsontiporn S and Premasthira C (1991) *Pennisetum* weed species in Thailand - their distribution and problems. *Report of the Tohoku Branch, the Crop Science Society of Japan* **34**:101-103.

Harris K M (1963) Assessments of the infection of guineacorn (*Sorghum vulgare*) by covered smut (*Sphacelotheca sorghi* (Link) Clinton) in northern Nigeria in 1957 and 1958. *Annals of Applied Biology* **51**:367-370.

Hassan N I, Osman A F and Rammah A M (1983) New varieties of Napier grass grown in Egypt. *World Review of Animal Production* **XIX,4**:35-40.

Hau B, Amorim L and Bergamin Filho (1993) Mathematical functions to describe disease progress curves of double sigmoid pattern. *Phytopathology* **83,9**:928-932.

Hawksworth D L, Kirk P M, Sutton B C and Pegler D N (eds) (1995) *Ainsworth & Bisby's Dictionary of the Fungi.* Eight Edition. CAB International, Wallingford, UK. pp. 616.

Henderson G R and Preston P T (1957) *Fodder Farming in Kenya.* East Africa Literature Bureau, Nairobi, Kenya. pp. 149.

Hindorf H (1972) Weed associations in sisal (*Agave sisalana*) and their control. *Berichte aus der Abteilung fur Herbologie an der Universitat Hohenheim* **3**:57-63.

Hirschhorn E (1949) Un nuevo metode de infection artificial con el carbon de la cana de azucar. *Revto Invest. agric. B. Aires* **3**:335-344.

Hirschhorn E (1973) New data on the smut attacking *Bromus* spp. in Argentina. *Boletin de la Sociedad Argentina de Botanica* **18**:56-64.

Holliday P (1989) *A Dictionary of Plant Pathology*. Cambridge University Press, UK. pp. 369.

Hollier C A, Groth D E, Rush M C and Webster R K (1993) Diseases of rice (*Oryza sativa* L). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/rice] 1998.

Holton C S, Hoffman J A and Duran R (1968) Variation in the smut fungi. *Annual Review of Phytopathology* **6**:213-242.

Horne C W and Frederiksen (1993) Diseases of sorghum (*Sorghum bicolor* (L) Moench). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/sorghum] 1998.

Horne P M and Blair G J (1991) Forage tree legumes IV. Productivity of leucaena/grass mixtures. *Australian Journal of Agricultural Research* **42,7**:1231-1250.

Horsfall J G and Dimond A E (1959) *Plant Pathology, Vol 1.* Academic Press, New York and London. pp. 674.

Hoy J W, Grisham M P and Chao C P (1991) Production of sori and dispersal of teliospores of *Ustilago scitaminea* in Louisiana. *Phytopathology* **81**:574-579.

Hoy J W, Zheng Jiaxie, Grelen L B and Geaghan J P (1993) Longevity of teliospores of *Ustilago scitaminea* in soil. *Plant Disease* **77(4)**:393-397.

Ibrahim K M and Kabuye C H S (1988) *An Illustrated Manual of Kenya Grasses*. FAO, Rome, Italy. pp. 312.

Ingold C T (1983) The basidium in *Ustilago. Transactions of the British Mycological Society* **81**:573-584.

Ingold C T (1984) Further studies on the basidium of *Ustilago. Transactions of the British Mycological Society* **83**:251-256.

Ingold C T (1985) The basidium of *Ustilago tragopogonis-pratensis. Transactions of the British Mycological Society* **84**:731-732.

Ingold C T (1986) The basidium of *Sphacelotheca sorghi. Transactions of the British Mycological Society* **87**:474-476.

Ingold C T (1987) Germination of teliospores in certain smut fungi. *Transactions of the British Mycological Society* **88**:355-363.

Ingold C T (1988) Teliospore germination in some species of Ustilaginaceae. *Transactions of the British Mycological Society* **91**:505-514.

Ingold C T (1989) Basidium development in some species of *Ustilago. Mycological Research* **93**:405-412.

Ingram W R (1958) The lepidopterous stalk borers associated with Graminae in Uganda. *Bulletin of Entomological Research* **49**:367-383.

Jaetzold R and Schmidt H (1983) *Farm Management Handbook of Kenya. Vol. IIB Central Kenya.* Ministry of Agriculture, Kenya/GTZ. pp. 739.

Jairajpuri M S and Siddiqi M R (1977) Observations on the nematode genera *Orientylus* and *Calvatylus* (Rotylenchoidinae:Hoplolaimidae) with descriptions of three new species. *Indian Journal of Nematology* **7,2**:101-111.

Jama B, Getahun A and Amare Getahun (1991) Fuelwood production from *Leucaena leucocephala* established in fodder crops in Kenya. *Agroforestry Systems* **16,2**:119-128.

James G L (1973) Smut spore germination on sugarcane internode surfaces. In: *Proceedings of the 47th Annual Congress, South African Sugar Technologists' Association*. pp. 179-180.

James W C (1971a) An illustrated series of assessment keys for plant diseases, their presentation and usage. *Canadian Plant Disease Survey* **51**:39-65.

James W C (1971b) A manual of disease assessment keys for plant diseases. *Publication, Canadian Department of Agriculture* No. 1458. pp. 80.

James W C (1983) Crop loss assessment. In: A Johnston and C Booth (eds) *Plant Pathologist's Pocketbook.* Commonwealth Agricultural Bureaux. Slough, UK. pp. 130-143.

Javier E Q (1969) The phenology, flowering habits and mode of reproduction of *Pennisetum purpureum* Schumach., *Bracharia mutica* (Forsk.) Stapf and *Panicum maximum* Jacq. PhD Thesis, Cornell University. pp. 244.

Jennersten O (1988) Insect dispersal of fungal disease: effects of *Ustilago* infection on pollinator attraction in *Viscaria vulgaris. Oikos* **51,2**:163-170.

Johnston A and Booth C (1983) *Plant Pathologist's Pocketbook.* Commonwealth Agricultural Bureaux. Slough, UK. pp. 439.

Jones D G (1987) *Plant Pathology.* Oxford University Press, Milton Keynes, UK. pp. 191.

Joshi L M, Wilcoxson R D, Gera S D and Chatterjee S C (1974) Preservation of fungal cultures in liquid air. *Indian Journal of Experimental Biology* **12,6**:598-599.

Julian A M (1995) Epidemiology and control of the sorghum foliar pathogens. Final Technical Report. Natural Resources Institute, Chatham, UK.

Juangbhanich P and Wangwon S (1983) Effect of temperature and some substrates on teliospore germination of sugarcane smut and inoculation methods on infection by *Ustilago scitaminea*. *Katsetart Journal* **17,2**:45-52.

Kagan F and Stuziński A (1967) Wycena strat powodowanych przez choroby zbóż z rodzaju Tilletia i Ustilago w Polsce w latach 1961-1965. (Assessment of losses caused by Tilletia and Ustilago in Poland 1961-1965). *Prace Naukowe Instytutu Ochrony Roślin* **9(2)**:175-210.

Kaiser W J, Ndimande B N and Hawksworth D L (1979) Leaf scorch disease of sugarcane in Kenya caused by a new species of *Leptosphaeria*. *Mycologia* **71,3**:479-492.

Karanja G M (1981) Bana grass. *Kenya Farmer* **3**:50-52.

Kendrick E L and Holton C S (1960) Differential longevity of teliospores of pathogenic races of *Tilletia caries* and *T. foetida*. *Phytopathology* **50**:51-54.

Kennedy D (1978) *Recipes from the Regional Cooks of Mexico*. Harper and Row, New York. pp. 288.

Keshan Singh, Budhraja T R and Lal A (1975) An evaluation of the negative pressure technique for smut inoculation in sugarcane. *Indian Journal of Agricultural Science* **45(9)**:403-404.

Krambias A, Zyngas J P and Shiakides T (1973) Outbreaks and new records. *FAO Plant Protection Bulletin* **21,3**:64-67.

Kranz J (1988) Measuring plant disease. In: J Kranz and J Rotem (eds) *Experimental Techniques in Plant Disease Epidemiology*. Springer-Verlag, Berlin. pp. 35-50.

Kratka J and Ujevic I (1974) Prolongation of chlamydospore germination of *Ustilago nuda* (Jens.) Rostr. *Ochrana Roślin* **10,2**:97-101.

Kung'u J N and Boa E R (1997) *Kenya Checklist of Fungi and Bacteria on Plants and other Substrates.* KARI/CAB International/DFID. pp. 96.

Kung'u J N and Waller J M (in press) Occurrence of smut of Napier grass caused by *Ustilago kamerunensis* H Sydow and Sydow in Kenya. *International Journal of Pest Management*.

Kuniata L S and Young G R (1992) The biology of *Lepidiota reuleauxi* Brenske (Coleoptera:Scarabaeidae), a pest of sugarcane in Papua New Guinea. *Journal of the Australian Entomological Society* **31,4**:339-343.

Langdon R F N and Fullerton R F A (1975) Sorus ontogeny and sporogenesis in some smut fungi. *Australian Journal of Botany* **23**:915-930.

Ledermann C (1908) Herbarium record 162103, Systematic Botany and Mycology Laboratory, USDA, Maryland, USA. [http://nt.ars-grin.gov/fungaldatabases/fungushost] 1998.

Lenné J M (1989) Evaluation of biotic factors affecting grassland production - history and prospects. In: *Proceedings of the XVIth International Grassland Congress, Nice, France, October 1989*. pp. 1811-1815.

Lenné J M (1990) *A World List of Fungal Diseases of Tropical Pasture Species*. Phytopathological Papers No. 31. CAB International/CIAT. Wallingford, UK. pp. 162.

Lenné J M and Sonoda R M (1990) Tropical pasture pathology: a pioneering and challenging endeavour. *Plant Disease* **74**:945-951.

Lenné J M and Trutmann P (eds) (1994) *Diseases of Topical Pasture Plants.* CAB International, Wallingford, UK. pp. 404.

Le Pelley R H (1959) *Agricultural insects of East Africa*. East Africa High Commission, Nairobi, Kenya. pp. 307.

Leu L S (1972) Culmicolous smut of sugarcane in Taiwan III. Germination and storage of teliospores, and compatibility of *Ustilago scitaminea* Sydow. *Report of the Taiwan Sugar Experimental Station* **56**:37-48.

Leu L S, Wang Z N and Hsieh W H (1974) Leaf blight of sugarcane in Taiwan V. Host range and hypersensitive reaction. *Report of the Taiwan Sugar Research Institute* **63**:59-68.

Lindeberg B (1959) *Ustilaginales of Sweden*. Symb. Bot. Upsal. **16(2)**:1-75.

Line R F (1988) Quarantines for control of flag smut of wheat: are they effective or necessary?In: *Proceedings of Bunts and Smuts of Wheat: an International Symposium.* 17-20 August 1997, Raleigh, North Carolina, USA.

Ling L (1940) Factors affecting spore germination and growth of *Urocystis occulta* in culture. *Phytopathology* **30**:579-591.

Liou T N (1949) Sur l'existance d'une nouvelle famille des Ustilaginales. *Contr. Inst. Bot. Natl. Acad. Peiping* **6**:37-47.

Lloyd H L and Pillay M (1980) The development of an improved method for evaluating sugarcane for resistance to smut. *Proceedings of the South African Technologists' Association*. June 1980. pp. 168-172.

Lowe A, Hanson J and Teale A (in preparation) Molecular characterisation of Napier grass using random amplified polymorphic DNA (RAPD) markers.

Lowther C V (1950) Chlamydospore germination in physiologic races of *Tilletia caries* and *Tilletia foetida*. *Phytopathology* **40**:590-603.

Luc M and Guiran G de (1960) Les nématodes associés aux plantes de l'ouest Africain. Liste preliminaire. *Agron. Trop. Nogent.* **15**:434-449.

Lusweti F N (1988) Agronomic aspects in the management of headsmut of Napier grass. In: *Proceedings of the 2nd Napier Grass Workshop.* KARI/DFID NARP II, Nairobi, Kenya. pp. 29-31.

Madden L V, Louie R, Abt J J and Knoke J K (1982) Evaluation of tests for randomness of infected plants. *Phytopathology* **72**:195-198.

Maher C (1936) Elephant grass (*Pennisetum purpureum*) as a cattle fodder in Kenya. *East African Agricultural and Forestry Journal* **12**:305-317.

Malaguti G, Carrasquel S R and Gallardo A (1972) *Pyricularia grisea* on elephant grass and other Graminae. *Agronomia Tropical* **22,3**:323-329.

Mansour I M, Abdou Y A, Moursy M A and Abdel Fattah M N D (1991) Interaction of soil moisture and soil fungistasis on infectivity of *Ustilago scitaminea* teliospores. *Egyptian Journal of Agricultural Research* **69,3**:625-635.

MapInfo (1995) MapInfo v 4.1. MapiInfo Corporation, Troy, NY, USA.

Martin G C (1956) The common root-knot nematode and crop rotation. *Rhodesian Farmer.* pp. 20.

Martins C R F and Kitajima E W (1993) A unique virus isolated from elephant grass. *Plant Disease* **77,7**:726-729.

Mathre D E (1993) Diseases of barley (*Hordeum vulgare* L amend Bowden). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/barley] 1998.

Mathur R S, Jain J S and Bajpai G K (1965) Assessment of loss due to grain smut of jowar millet (*Sorghum vulgare*) in India. *Plant Disease Reporter* **49**:164-166.

Matias C and Matias Y (1995) Effect of support on seed production in *Teramnus labialis* cv. Semilla Clara I. Choice of supports. *Pastos y Farrrajes* **18,1**:51-57.

McDonald J (1929) A list of plant diseases in Kenya colony. *Department of Agriculture Kenya Bulletin No. 5.*

McDonald J (1936) A revised list of plant diseases in Kenya colony. *East African Agricultural Journal* **I**:463-468.

McDonald J (1937) *Coffee in Kenya*. Government Printer, Nairobi, Kenya. pp. 210.

McSorley R, Hammond L C and Dunn R A (1989) Nematodes associated with *Pennisetum purpureum* and *Saccharum* sp. in Florida. *Nematropica* **19,1**:29-37.

Melle H A (1918) Agricultural grasses and their control. *Pretoria Dep. Agric. Bull.* **5**.

Menezes M de and De-Menezes M (1978) Notes on the oviposition habits and the host plants of *Apogonalia grossa* (Signoret, 1854) (Homoptera:Cicadelidae:Cicadellinae). *Revista Brasileira de Entomologia* **22,2**:61-64.

Mengistu Hulluka, Fikru Haile and Sewalem Amogne (1993) Survey of diseases, insect pests and weeds at the ILCA Herbage Seed Unit Farm, Debre Zeit. *ILCA Internal Report*. pp. 14.

Michieka R W and Akobundu I O (1984) Control of *Pennisetum purpureum* Schumach. in no-tillage maize (*Zea mays* L). In: *Proceedings of the Ninth Annual Conference of the Weed Science Society of Nigeria*. pp. 75-79.

Mohamed-Saleem M A and Berhe K (1994) Diseases of Tropical Pasture Plants in Sub-Saharan Africa. In: Lenné J M and Trutmann P (eds)(1994) *Diseases of Topical Pasture Plants.* CAB International, Wallingford, UK. pp. 329-345.

Mohanraj D, Padmanaban P and Alexander K C (1987) Effect of inoculation method on early expression of sugarcane smut. *Sugar Cane* **5**:7-8.

MoLD (undated) *The Management of Napier Grass.* Ministry of Livestock Development, Zero Grazing Series Vol. 2, Nairobi, Kenya. pp. 18.

Momol M T, Purdy L H and Schmidt R A (1990) Disease gradients of sugarcane smut in Florida. *Sugarcane* **2**:3-5.

Moore M B (1936) A method for inoculating wheat and barley with loose smuts. *Phytopathology* **26**:397-400.

Moore R T (1978) Taxonomic significance of septal ultrastructure with particular reference to the jelly fungi. *Mycologia* **70**:1007-1024.

Moore R T (1996) An inventory of the phylum Ustomycota. *Mycotaxon* **59**:1-31.

Mordue J E M and Ainsworth G C (1984) *Ustilaginales of the British Isles.* CAB International, Wallingford, UK.

Mordue J E M and Waller J M (1981) *Urocystis agropyri*. CMI Descriptions of Pathogenic Fungi and Bacteria, No. 716. CMI, Kew, UK.

Mtisi E and Milliano W A J de (1993) False mildew on pearl millet and other hosts in Zimbabwe. *East African Agricultural and Forestry Journal* **59(2)**:145-153.

Mureithi J G, Tayler R S and Thorpe W (1995) Productivity of alley farming with leucaena (*Leucaena leucocephala* Lam. de Wit) and Napier grass (*Pennisetum purpureum* K Schum.) in coastal lowland Kenya. *Agroforestry Systems* **31**:59-78.

Musembi F and Nyanyu G (1998) Results of PRAs on Napier grass headsmut in Kiambu and Thika Districts. In: *Proceedings of the 2nd Napier Grass Workshop*. KARI/DFID NARP II, Nairobi, Kenya. pp. 26-27.

Mutisya D N and Lado C (1991) Some socio-economic factors behind roadside farming in Kiambu District, Kenya. *Journal of East African Research and Development* **21**:107-127.

Nakagawa J, Prochnow L I, Bull L T and Boas R L V (1992) Effects of organic compost on a lettuce (*Lactuca sativa* L.) crop. Part I. *Cientifica Jaboticabal* **20,1**:173-180.

Nasr I A (1977) Standardisation of inoculation techniques for sugarcane smut disease. *Sugarcane Pathologists' Newsletter* **18**:2-5.

Nattrass R M (1941) Notes on plant diseases. *East African Agricultural Journal 1977-1980* **7**:56.

Nattrass R M (1961) Host lists of Kenya fungi and bacteria. *Department of Agriculture, Kenya. Mycological Papers, No. 81.* pp. 46.

Njuguna J G M, Gordon D T and Louie R (1997) The role of Napier grass (*Pennisetum purpureum*) in the epidemiology of maize streak disease in Kenya. In: P O Fungah and G C O Mbadi (eds) In: *Focus on Agricultural Research for Sustainable Development in a Changing Economic Environment. Proceedings of the 5th KARI Scientific Conference.* Kenya Agricultural Research Institute, Nairobi, Kenya. pp. 25-37.

Nye G W (1937) Preliminary notes on the use of elephant grass as a fallow crop in Buganda. *East African Agricultural Journal* **3(3)**:186-190.

Oberwinkler F, Bandoni R J, Blanz P, Deml G and Kisimova-Horovitz L (1982) Graphiolales: basidiomycetes parasitic on palms. *Pl. Syst. Evol.* **140**:251-277.

Olive L S (1968) An unusual new Heterobasidiomycete with *Tilletia*-like basidia. *J. Elisha Mitchell Sci. Soc.* **84**:261-266.

Olufolaji D B (1987) An alternative host of *Ustilago scitaminea*, the sugarcane smut fungus. *Sugarcane* 1987 Spring Supplement: 30-32.

Ondieki J J (1973) Host lists of Kenya fungi and bacteria (a ten year supplement 1961-1970). *Special Issue of the East African Agricultural and Forestry Journal.* pp. 31.

Onyango T A and Kevelenge J E (1987) Forage production and utilization. *Bulletin of the International Dairy Federation* **221**:14-22.

Orsenigo J R (1977) Chemical control of ditchbank weeds in peat and sandy soil areas of South Florida. *Annual Research Report, Institute of Food and Agricultural Sciences, Florida, 1975.* pp. 252.

Padmanaban P, Alexander K C and Shanmugan N (1988) Mechanism of smut resistance in sugarcane. *Sugar Cane* **6**:14-16.

Palacpac O S (1985) Philippines-Japanese venture finds market for elephant grass is booming. *Agribusiness Worldwide.* July/August 1995. pp. 19-24.

Pandey D K and Gautam P L (1989) Comparison of inoculation techniques for loose smut of wheat. *Plant Disease Research* **4,2**:167-169.

Patil S P and Veeresh G K (1984) Description of larvae of *Hybosorus orientalis* Westwood (Coleoptera:Scarabaeidae). *Journal of Maharashtra Agricultural Universities* **9,1**:110-111.

Pauvert P and Jacqua G (1974) A critical study of *Helminthosporium* spp. from *Panicum, Pennisetum* and sugarcane in Antilles. *Annales de Phytopathologie* **6,3**:285-296.

Payne R W, Lane P W, Ainsley A E, Bicknell K E, Digby P G N, Harding S A, Leech P K, Simpson H R, Todd A D, Verrier P J, White R P, Gower J C, Tunnicliffe-Wilson G and Paterson L J (1987) *Genstat 5 Reference Manual.* Oxford University Press, Oxford, UK. pp. 796.

PCPB (1997) List of pest control products registered by the Pest Control Products Board for use in Kenya. Pest Control Products Board, Nairobi, Kenya. pp. 11.

Perez J R, Castro M and Hernandez N (1981) Problems with elephant grass for the diagnosis of ratoon stunting disease in Cuba. *Ciensas de la Agricultura* **10**:114-116.

Persoon C H (1801) *Synopsis Methodica Fungorum.* Gottingae, Die rich. Pars. I and II, XXX. pp. 706.

Piepenbring M, Vánky K and Oberwinkler F (1996) *Aurantiosporium*, a new genus for *Ustilago subnitens. Plant Systematics and Evolution* **199,1-2**:53-64.

Pierrard G (1969) Injuriousness of Diplopods to cultivated plants in hot countries. *Coton et Fibres Tropicales* **24,4**:429-441.

Poate C D and Daplyn P F (1993) *Data for Agrarian Development.* Cambridge University Press, Cambridge, UK. pp. 387.

Poehlman J M (1945) A simple method of inoculating barley with loose smut. *Phytopathology* **35**:640-653.

Polhill R M (1982) *Flora of Tropical East Africa*. *Graminae*. A A Balkema, Rotterdam, The Netherlands. pp. 750.

Prain D (1934) Flora of Tropical Africa: Vol IX Graminae (Maydae - Paniceae). L Reeve, Ashford, UK. pp. 1132.

Pruett C J H and Colque-A E (1984) Fungal spore feeding Coleoptera in sugarcane smut caused by *Ustilago scitaminea* Syd. and head smut caused

by *Sphacelotheca reiliana* (Kühn) Clinton in Sudan grass *Sorghum sudanense* (Piper) Stampf in the Bolivian orient. *Entomology Newsletter* **16**:14-17.

Purseglove J W (1972) *Tropical Crops - Monocotyledons*. ELBS/Longman, UK. pp. 607.

Ragab M M M (1994) Antagonism between epiphytic microorganisms and *Ustilago maydis* causing common smut of maize. *Egyptian Journal of Phytopathology* **22,1**:17-37.

Rampersad E M and Brathwaite C W D (1985) Evidence to support the involvement of physiological factors in the smut resistance of nine varieties of sugarcane grown in Trinidad and Tobago. In: *Proceedings of the 1985 Meeting of West Indies Sugar Technologists, March 1985, Trinidad and Tobago.* pp. 762-775.

Rao G P, Mathuresh Singh, Verma K P, Singh H N and Singh M (1990) Munj grass (*Saccharum munja* Roxb.), a natural host of sugar cane smut. *National Academy Science Letters* **13**:3.

Rishi N, Bhargava K S and Joshi R D (1973) Perpetuation of mosaic disease of sugarcane. *Annals of the Phytopathological Society of Japan* **39,4**:361-363.

Rivera N, Garcia A and Gil C L (1980) Pathotype characterisation of *Erwinia chrysanthemi* from banana and maize. *Ciencia y Tecnica en la Agricultura, Proteccion de Plantas* **3,2/3**:47-60.

Robinson R A (1959) Sugarcane smut. *East African Agricultural Journal* **24**:240-243.

Robinson R A (1960) Notes on Kenya agriculture VIII: important plant diseases. *East African Agricultural Journal* **25**:131-146.

Robinson S W (1979) Leaf-cutting ant control schemes in Paraguay, 1961-1977. Some failures and some lessons. *PANS* **25,4**:386-390.

Roche B M, Alexander H M and Maltby A D (1995) Dispersal and disease gradients of anther-smut infection of *Silene alba* at different life stages. *Ecology* **76(6)**:1863-1871.

Rose D J W (1973) Field studies in Rhodesia on *Cicadulina* spp. (Homoptera: Cicadellidae) in relation to maize streak disease in Rhodesia. *Bulletin of Entomological Research* **62**:477-495.

Rott P, Chatenet M, Granier M and Baudin P (1988) Leaf scald disease of sugarcane caused by *Xanthomonas albilineans* (Ashby) Dowson. II - three methods and host spectrum for the diagnosis of the pathogen in tropical Africa. *Agronomie Tropicale* **43,3**:244-251.

Roussel H F A (1806) *Flore du Calvados et des Terrains Adjacens, 2nd Edition.* Caen, France.

Russell L M and Etienne J (1985) A list of Aleyrodidae of the Island of Réunion. *Proceedings of the Entomological Society of Washington* **87,1**:202-206.

Sachan J N (1980) Evaluation of incidence of insect pests on hybrid Napier strains under different dosages of fertilizers. *Annals of the Arid Zone* **19,1/2**:82-91.

Salau O A, Opara Nadi O A and Swennen R (1992) Response of plantain to mulch on a tropical ultisol. Part I. Effect of different mulching materials on soil physical and chemical properties. *International Agrophysics* **6,1-2**:55-64.

Scarr M J (1988) Extended Napier grass smut disease survey. In: *Proceedings of the 2nd Napier Grass Workshop*. KARI/DFID NARP II, Nairobi, Kenya. pp. 2-4.

Scheibelreiter G and Inyang P (1974) *Epilachna similis* Muls. (Coleoptera:Coccinellidae), a minor pest on maize in Ghana. *Ghana Journal of Agricultural Science* **7(2)**:75-79.

Schoningh E (1985) The effect of mulch on yield and factors of soil fertility in the eastern Amazonas region of Brazil. *Giessener Beitrage zur Entwicklungsforschung* **II**. pp. 189.

Seshadri K, Padmanaban P, Mihanraj D and Alexander K C (1985) A technique for inoculation of sugarcane seed with *Ustilago scitaminea. Indian Phytopathology* **38**:177-178.

Shepherd B M and Ferrer E R (1990) Sampling insects and diseases in rice. In: *Crop Loss Assessment in Rice. International Workshop on Crop Loss Assessment to Improve Pest Management in Rice and Rice Based Cropping Systems in South and South-east Asia.* International Rice Research Institute, Los Banos, Philippines. pp. 107-130.

Shivas R G and Vánky K (1997) New smuts (Ustilaginales) on grasses in Western Australia. *Mycological Research* **101,7**:835-840.

Shurtleff M C, Edwards D I, Noel G R, Pedersen W L and White D G (1993) Diseases of corn or maize (*Zea mays* L). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/corn] 1998.

Siagian N and Sumaradji (1989) Forage grasses as alternative cover crops for young rubber. *Bulletin Perkaretan* **7,3**:75-79.

Siddiqi M R (1979) Seven new species in the new nematode subfamily Duosulciinae (Tylenchidae), with proposals for *Duosulcius* gen. n., *Zanenchus* gen. n. and *Neomalenchus* gen. n. *Nematologica* **25,2**:215-236.

Siddiqi M R (1980) Taxonomy of the plant nematode superfamily Hemicycliophoroidea, with a proposal for Criconematina, new suborder. *Revue de Nématologie* **3,2**:179-199.

Silva J L and Neves P M O J (1984) Occurrence and control of *Mocis latipes* (Guem., 1952) in tropical pastures in the north of Parana. *Semina* **5,16**:102.

Simmonds N W (1994) Some speculative calculations on the dispersal of sugarcane smut disease. *Sugar Cane* **1**:2-5.

Singh B P and Misra A K (1974) Effect of chemicals on flowering of chrysanthemum stunt virus-infected plants. *Proceedings of the Indian Academy of Science* **80,6**:267-274.

Singh R and Chaudhary J P (1987) Relative suitability of some graminaceous host plants to *Mythimna lorayi* (Dup.) (Lepidoptera: Noctuidae). *Zeitschrift fur Angewandte Zoologie* **74,3**:377-384.

Singh R and Chaudhary J P (1988) Effect of plant species on post-embryonic development and survival of armyworm (*Mythimna separata*). *Indian Journal of Agricultural Sciences* **58,11**:873-875.

Singh S D (1988) Recovery resistance to downy mildew in pearl millet. *Plant Disease* **72,5**:425-428.

Singh S D (1989) First report of recovery of sorghum from downy mildew in Zimbabwe. *Plant Disease* **73,12**:1020.

Singh Sandhu G (1975) Seasonal movement of the painted bug, *Bagrada cruciferarum* Kirkaldy, from cruciferous plants to graminaceous plants and its occurrence as a serious pest of maize, sorghum and pearl millet during spring in the Punjab. *Indian Journal of Entomology* **37,2**:215-217.

Sinha O K and Kishan Singh (1983) Antagonistic activity of *Fusarium moniliforme* var. *subglutinans* against sugarcane smut. *Indian Phytopathology* **36,1**:92-94.

Sistachs M, Barrientos A and Padilla C (1990) Intercropping of kenaf (*Hibiscus cannabinus*) in guinea grass (*Panicum maximum*), star grass (*Cynodon nlemfuensis*) and king grass (*Pennisetum purpureum*) establishment. *Cuban Journal of Agricultural Science* **24,3**:327-333.

Skerman P J and Riveros F (1990) *Tropical Grasses.* FAO, Rome, Italy.

Slinkard A E and Elliot F C (1954) The effect of bunt incidence on the yield of wheat in eastern Washington. *Agronomy Journal* **46**:439-441.

Smiley R W (1993) Diseases of turf grasses. *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/turfgrass] 1998.

Snowden J D (1930) Herbarium record 162104, Systematic Botany and Mycology Laboratory, USDA, Maryland, USA. [http://nt.ars-grin.gov/fungaldatabases/fungushost] 1998.

Spooner B M (1991) *Puccinie/lia maritima* - a new host for the smut fungus *Ustilago striiformis. Kew Bulletin* **32,1**:8.

Sprague R (1950) *Diseases of Cereals and Grasses in North America*. Ronald Press, New York. pp. 538.

Staal S J, Chege L, Kenyanjui M, Kimari A, Lukuyu B, Njubi D, Owango M, Tanner J, Thorpe W and Wambugu M (1997) *Characterisation of Dairy Farming Systems Supplying the Nairobi Milk Market.* KARI/MoA/ILRI Collaborative Research Project Report. Revised 1998. pp. 85.

Stapf O (1934) Pennisetum. In: D Prain (1934) Flora of Tropical Africa: Vol IX Graminae (Maydae - Paniceae). L Reeve, Ashford, UK. p. 955.

Steindl D R L (1974) Methods used in the identification of ratoon stunting disease. *Cane Growers' Quarterly Bulletin* **38,1**:23-25.

Stewart A V (1992) Grasslands Gala grazing brome (*Bromus stamineus*) - a new dryland pasture grass. *New Zealand Journal of Agricultural Research* **35,3**:349-353.

Sudrajat R and Hartadi D (1992) Agricultural waste product processing with particular reference to oil palm waste. *Jurnal Penelitian Hasil Hutan* **10,6**:197-208.

Sutherland J A, Kibata G N and Farrell G (1996) *Field Sampling Methods for Crop Pests and Diseases in Kenya.* KARI/ODA Crop Protection Project, Nairobi, Kenya. pp. 58.

Suzuki S, Maffia L A and Cruz Filho J (1994) Sobrevivência de teliosporos de *Ustilago scitaminea* no solo. *Fitopatologia brasil* **19(3)**:442-448.

Swennen R and Wilson G F (1985) *Flemingia congesta* as a live cover plant for plantain. *Banana Newsletter* **8**:17-18.

Swynnerton R J M (1949) Some problems of the Chagga on Kilimanjaro. *East African Agricultural Journal* **14**:117-132.

Sydow H and Sydow P (1910) Fungi africani novi. *Bot. Jahrb.* (Engler) **45**:262.

Sylvester J A (1990) Napier grass control at Kaheka Sugar Company. *Hawaiian Sugar Technologists 48th Annual Conference Reports*: A42-A43.

Tachibana H and Duran R (1966) Comparative survival of teliospores and mycelia of the onion smut fungus in soil. *Phytopathology* **56**:136-137.

Tapke V F (1948) Prolonging viability of spores and mycelium of the barley loose smut, *Ustilago nuda*. *Phytopathology* **38**:25.

Tarr S A J (1955) *The Fungi and Plant Diseases of the Sudan.* Commonwealth Mycological Institute, Kew, UK. pp. 127.

Tcaceno F A and Lance G N (1992) Selection of morphological traits for characterisation of elephant grass accessions. *Tropical Grasslands* **26**:145-155.

Teng P S (ed) (1987) *Crop Loss Assessment and Pest Management.* The American Phytopathological Society, St Paul, Minnesota, USA. pp. 270.

Terrell E E and Batra L R (1982) *Zizania latifolia* and *Ustilago esculenta*: a grass-fungus association. *Economic Botany* **36(3)**:274-285.

Terry P J (1984) *A Guide to Weed Control in East African Crops.* UK Overseas Development Administration/Kenya Literature Bureau. pp. 186.

Thaxter R (1890) The smut of onions (*Urocystis cepulae* Frost). *Connecticut Agricultural Experimental Station Annual Report*:127-154.

Thomas G V (1988) Vesicular-arbuscular mycorrhizal symbiosis in coconut

(*Cocos nucifera*) in relation to the root (wilt) disease and intercropping or mixed cropping. *Indian Journal of Agricultural Sciences* **57,2**:145-147.

Thomson J (1885) *Through Masai Land: A Journey of Exploration among the Snowclad Volcanic Mountains and Strange Tribes.* Sampson Low, Marston, Searle and Rivington. London, UK. pp. 583.

Thrall P H, Biere A and Uyenoyama M K (1995) Frequency-dependent disease transmission and the dynamics of the *Silene-Ustilago* host-pathogen system. *American Naturalist* **145(1)**:43-62.

Thrall P H and Jarosz A M (1994) Host-pathogen dynamics in experimental populations of *Silene alba* and *Ustilago violacea*. Experimental tests of theoretical models. *Journal of Ecology* **82(3)**:561-570.

Tillet M (1755) Dissertation on the cause of the corruption and smutting of the kernels of wheat in the head, and the means of preventing these untoward circumstances. Bordeaux, France (Translated from the French by H B Humphrey). American Phytopathological Society (1970) *Phytopathological Classics* **5.** pp.191.

Tiley G E D (1969) Elephant grass. *Kawanda Research Station Report.* Kawanda, Uganda.

Tomlin C D S (ed)(1997) *The Pesticide Manual.* 11th edition. British Crop Protection Council, Farnham, UK. pp.1606.

Tothill J D (1940) *Agriculture in Uganda*. Oxford University Press, Oxford, UK.

Tozzetti G T (1767) True nature, causes and sad effects of the rust, the bunt, the smut and other maladies of wheat and of oats in the field. Florence, Italy (Translated from the Italian by L R Tehon). American Phytopathological Society (1952) *Phytopathological Classics* **9.** pp. 139.

Tulasne L-R and Tulasne C (1847) Mémoire sur les Ustilaginées comparées Urédinées. *Ann. Sci. Nat. Bot.* **Sér. 3, 7**:12-127.

Turgeon A J, Beard J B, Martin D P and Meggitt W F (1974) Effects of successive applications of pre-emergence herbicides on turf. *Weed Science* **22,4**:349-352.

Vaishnav M U, Sabalpara A N and Khandar R R (1992) Mycoparasitism on sugarcane smut (*Ustilago scitaminea* Syd.) by four fungi. *Indian Journal of Mycology and Plant Pathology* **22,2**:142-145.

Vaishnav M U and Sethi C L (1977) Reaction of some graminaceous crops to *Meloidogyne incognita* and *Tylenchorhynchus vulgaris*. *Indian Journal of Nematology* **7**:176-177.

Valk Y S (1990) Review report of the DEAF surveys during 1989. NDDP/M41/200. Ministry of Livestock Development, Nairobi, Kenya.

Vanderwalle R (1942) Note sur la biologie d'*Ustilago nuda tritici* Schaf. *Bull. Inst. Agron. Gembloux* **11**:103-113.

van der Wouw M, Hanson J and Luethi S (in press) Morphological and agronomic characterisation of a collection of Napier grass (*Pennisetum purpureum* and *P. purpureum* x *P. glaucum*). *Tropical Grasslands.*

Vánky K (1987) *Illustrated Genera of Smut Fungi*. Gustav Fischer Verlag, Stuttgart and New York. pp. 159.

Vánky K (1991) Taxonomical studies on Ustilaginales 8. *Mycotaxon* **41,2**:483-495.

Vánky K (1992) Taxonomical studies on Ustilaginales 9. *Mycotaxon* **43**:417-425.

Vánky K (1993) Taxonomical studies on Ustilaginales 10. *Mycotaxon* **48**:27-44.

Vánky K (1994) Taxonomical studies on Ustilaginales 11. *Mycotaxon* **51**:152-174.

Vánky K (1995a) Taxonomical studies on Ustilaginales 12. *Mycotaxon* **54**:215-238.

Vánky K (1995b) Taxonomical studies on Ustilaginales 13. *Mycotaxon* **56**:197-216.

Vánky K (1996a) The genus *Macalpinomyces* (Ustilaginales). *Mycotaxon* **59**:115-126.

Vánky K (1996b) Taxonomical studies on Ustilaginales 14. *Mycotaxon* **59**:89-113.

Vánky K (1997) New Ustilaginales from Australia. *Mycotaxon* **62**:151-174.

Vargas F O and Pajuelo C (1973) Effect of *Meloidogyne* sp. (Nematoda:Heteroderidae) on some species of forage grass. *Anales*

Cientificos **11, 3-4**:205-218.

von Arx J A (1967) *Pilzunde: ein kurzer Abriss der Mykologie unter besonder Berucksichtigung der Pilze in Reinkultur.* Lehre: Cramer.

Wadhi S R, Misra S S and Verma B R (1973) First record of *Rhopalosiphum maidis* (Fitch) as a pest of Napier grass in India. *Indian Journal of Entomology* **35,3**:273-274.

Waggoner P E (1968) Weather and the rise and fall of fungi. In: W P Lowry (ed) *Biometeorology.* Oregon State University Press, Corvallis, USA. pp. 45-66.

Waller J M (1967) Varietal resistance to sugarcane smut in Kenya. *East African Agricultural and Forestry Journal* **32**:399-403.

Waller J M (1969) Sugarcane smut (*Ustilago scitaminea*) in Kenya. I Epidemiology. *Transactions of the British Mycological Society* **52(1)**:139-151.

Waller J M (1970) Sugarcane smut (*Ustilago scitaminea*) in Kenya. II Infection and resistance. *Transactions of the British Mycological Society* **54(3)**:405-414.

Wang C S (1936) Viability and longevity of chlamydospores of *Ustilago crameri*. *Phytopathology* **26**:1086-1087.

Waraiich K S (1989) Resistance testing techniques for whip-smut disease of sugarcane. *Indian Journal of Mycology and Plant Pathology* **19,2**:227-229.

Warham E J (1990) A comparison of inoculation techniques for assessment of germplasm susceptibility to Karnal bunt (*Tilletia indica*) disease of wheat. *Annals of Applied Biology* **116**:43-60.

Watson A J (1971) *Foreign Bacterial and Fungus Diseases of Food, Fodder and Fibre Crops.* Agric. Res. Serv. USDA. pp.111.

Watson L (1972) Smuts on grasses: some general implications of the incidence of Ustilaginales on the genera of Graminae. *The Quarterly Review of Biology* **47**:46-62.

Websdane K A, Sivasithamparam K, Dixon K W and Meney K A (1994) *Tolyposporium restonium* (*comb. nov.*) on *Alexgeorgea* species (Restionaceae) in Western Australia. *Mycotaxon* **51**:471-477.

Weise M V, Murray T D and Forster R L (1993) Diseases of wheat (*Triticum* spp.L.). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/wheat] 1998.

Whiteman P C (1980) *Tropical Pasture Science.* Oxford University Press, UK. pp. 392.

Whittle A M (1982) Yield loss in sugarcane due to culmicolous smut infection. *Tropical Agriculture* **59(3)**:239-242.

Williams C N, Chew W Y and Rajaratnam J A (1980) *Tree and Field Crops of the Wetter Regions of the Tropics*. ELBS/Longman, UK. pp. 262.

Wilson J P and Claflin L (1996) Diseases of pearl millet (*Pennisetum glaucum* (L) R Br). *American Phytopathological Society* [http://www.scisoc.org/resource/commom/names/pearlm] 1998.

Woodward K R, Prine G M and Bates D B (1991) Silage characteristics of elephantgrass as affected by harvest frequency and genotype. *Agronomy Journal* **83**:547-551.

Young G R and Kuniata L S (1992) Life history and biology of *Sesamia griscens* Walker (Lepidoptera:Noctuidae), a sugarcane borer in Papua New Guinea. *Journal of the Australian Entomological Society* **31,3**:199-203.

Zaheruddeen S M and Rao P S P (1988) Ovipositional host status of some weeds, wild rice (*Oryza* species) and cultivated crop plants for *Nilaparvata lugens. Indian Journal of Agricultural Sciences* **58,8**:659-661.

Zambettakis C H (1967) Reflexions sur la systematique des Ustilaginales. *Rev. Mycol.* **31**:384-392.

Zambettakis C H (1970) Recherches sur les Usatilaginales d'Afrique. *Bulletin Trimestriale de la Société Mycologique de France* **86**:545-546.

Zundel G L (1953) *The Ustilaginales of the World*. State College, PA; The Pennsylvania State College, College of Agriculture (Contribution No.176 from the Department of Botany). pp. 410.

APPENDIX 1 Descriptions of *Ustilago kamerunensis*

Sydow H and Sydow P (1910)

Ustilago kamerunensis Syd. n. sp.; soris in inflorescentiis evolutis easque omnino destruentibus, atris,; sporis globosis, levibus, brunneolis, 6-8µ diam.
Kamerun: Djutitsa's, an Inflorescenzen eines *Pennisetum* (Ledermann, Dez. 1980).

[translation: *Ustilago kamerunensis* Syd. n. sp.; sorus forms in the inflorescence which is completely destroyed, burnt black; spores globular, light, brownish, 6-8µ diam. Cameroon: Djutitsa's, on inflorescence of *Pennisetum* (Ledermann, Dez. 1980).]

Zundel G L (1953)

Sori in the ovaries, entirely destroying the inflorescence, transforming it into a dark brown, powdery spore-mass which dissipates leaving a naked rachis; spores globose, rarely subglobose, regular, thin walled, light reddish brown, 6-8µ diam., smooth.

Type host and locality: On *Pennisetum* sp., Djujitsa's, Cameroon, Afr.

On Poaceae: *Pennisetum purpureum* Schum.: Uganda. *P.* sp.: Cameroon.

(This seems to be largely a translation from Sydow and Sydow (1910))

Zambettakis C H (1970)

Ustilago kamerunensis **Sydow H. et P. (1910) (I).**
sur *Pennisetum purpureum* Schum., Uganda;
sur *Pennisetum* sp., Cameroun (Djutitsa).

PARTIES DE LA PLANTE ATTAQUEÉS: Ovaires, inflorescence. L'inflorescence est entièrment détruite.

SORES transformant l'inflorescence en une masse de spores poudreuse, brun foncé, et laissant un rachis nu.

TÉLIOSPORES sphériques, brun-rougeâtre clair, régulières, 6-8µ, lisses.

MEMBRANE SPORALE mince.

[translation: ***Ustilago kamerunensis*** **Sydow H. et P. (1910) (I).**
on *Pennisetum purpureum* Schum., Uganda;
on *Pennisetum* sp., Cameroon (Djutitsa).

PARTS OF THE PLANT ATTACKED: ovaries, inflorescence. The inflorescence is completely destroyed.

SORUS transforms the inflorescence into a mass of powdery spores, deep brown, and leaving a naked rachis.

TELIOSPORES spherical, reddish brown, regular, 6-8µ diam, smooth.

SPORE MEMBRANE thin.]

(This appears to be taken from the Sydow and Sydow (1910) and Zundel (1953) references)

APPENDIX 2

Postal questionnaire on early flowering and smut on Napier grass

1. Has Napier grass early flowering and/or smut been noticed in your district/region?

2. Is the disease always associated with early flowering?

3. When was it first noticed (year)?

4. Where was it first noticed (division, location, sub-location or village if possible).

5. Does the disease seem to be spreading?

6. What do farmers think is the method of spread?

   a) spontaneous
   b) through cuttings (local or from other areas)
   c) through animal manure
   d) any other method

7. What proportion of farmers are aware of the problem?

8. Are farmers trying any control measures?

9. What control measures are they trying and how successful are they?

10. Which varieties of Napier grass do farmers grow and are they all affected by smut disease?

APPENDIX 3

The agro-ecological zone system for land use classification.

Agro-ecological zones were established by FAO in 1978 (*op. cit.*), and modified for Kenya by Jaetzold and Schmidt (*op. cit.*) to provide a more differentiated system for farmers, showing yield probabilities and risks.

The zone groups (TA - tropical alpine, UH - upper highland, LH - lower highland, UM -upper midland, LM - lower midland, L - lowland, IL - inner lowland and CL - coastal lowland) are temperature belts defined according to the maximum temperature limits within which the main crops in the country can flourish. The highest zone is high altitude rough grazing. The threshold values of annual mean temperatures were supplemented by limiting factors such as mean minimum temperature and frost.

The main zones (0 [perhumid, 1[humid], 2 [subhumid], 3 [semi-humid], 4 [transitional], 5 [semi-arid], 6 [arid] and 7 [perarid]) are based on the zone groups' probability of meeting the temperature and water requirements of the leading crops. The names of the main zones refer to the potential leading crops eg LH1 is the tea-dairy zone, with a mean annual temperature of 15-18°C, mean minimum temperature of 8-11°C and normally no frost.

The main zones are divided into sub-zones according to the yearly

distribution and lengths of the growing periods with a probability factor of 60% ie the length of the growing period should be reached or exceeded in at least six years out of every ten.

APPENDIX 4

Sampling Napier plots AEZ sub-Location

Date Plot number Co-ordinates S

Altitude

E

Farmer Napier variety When planted?

When last cut?

Source of planting material Any plants flowering normally?

Has farmer seen smut on farm?

Adjacent crops Site; flat or sloping

Smut present?

Number of stools sampled

Plot boundaries sampled Plot area Number of stations

Comments

---

station number

1	17	33	49	65	81
2	18	34	50	66	82
3	19	35	51	67	83
4	20	36	52	68	84
5	21	37	53	69	85
6	22	38	54	70	86
7	23	39	55	71	87
8	24	40	56	72	88
9	25	41	57	73	89
10	26	42	58	74	90

11	27	43	99	75	91
12	28	44	60	76	92
13	29	45	61	77	93
14	30	46	62	78	94
15	31	47	63	79	95
16	32	48	64	80	96

APPENDIX 5

Napier pest and disease survey, January - June 1997

Plot details recommended napier density=18500 plants /ha

or orange
man mango
be beans
ca cabbage
lo loquat
ma maize
ba banana
fl flowers
pe peas
py pyrethrum
paw pawpaw
pas passion fruit
po potato
sp sweet potato
av avocado
co coffee
gr grevillea
rh rhubarb

dk don't know

flat or sloping

y yes
n no

ne neighbour

plot no.	date	altitude m	coordinates S	E	sub-location	soil type	area	site	Napier density plants per hectare	AEZ	adjacent crops	variety	when cut 1996/97	normal flowers?	source of napier	farmer	smut present in transect	smut present on plot	farmer seen smut?	age of stand	stool height	no. of stations	no. of stools	infested plot: no. of stools	infested plot: no. smut infested stools	infested plot: % smut infested stools
1	16-Jan-97	1951	01-14-37	36-42-02	Kanyariri	RB2	525	f	14716	UM3	ma, ba, lo	dk	Dec. '96	n	ne	Waithera	n	n	n	10+	0.5	44	35			
2	16-Jan-97	1984	01-14-18	36-41-35	Kanyariri	RB2	1738	s	13019	LH3	ma, ba	dk	Dec. '96	n	ne	Kabwe	n	n	n	2	0.5	81	57			
3	16-Jan-97	1987	01-14-09	36-41-19	Kanyariri	RB2	700	s	14338	LH3	lo, ba, be, ca	dk	Dec. '96	n	ne	Thindiu	n	n	n	10	0.5	40	31			
4	16-Jan-97	1810	01-13-56	36-41-46	Kanyariri	RB2	493	f	15961	LH3	ma, ba, lo, man, paw, pas	dk hairy	Oct. '96	n	ne	Kuria	n	n	n	4	2	51	44			
5	16-Jan-97	1850	01-13-41	36-41-53	Kanyariri	RB2	60	f	18500	LH3	ma, po, ba	dk smooth	Oct. '96	n	ne	Njunge	n	n	n	dk	1.5	20	20			
6	16-Jan-97	1943	01-13-47	36-41-52	Kanyariri	RB2	216	f	13455	LH3	ma, sp, ba	dk	dk	n	ne	Maina	n	n	n	20+	0.75	22	16			
7	22-Jan-97	1853	01-14-10	36-43-34	lower Kabete	RB2	612	s	11563	UM3	ma, ba	dk smooth	Dec. '96	n	farm in Muguga	Njeri	n	n	n	15	1	40	25			
8	22-Jan-97	1954	01-14-15	36-43-09	lower Kabete	RB2	460	f	15745	UM3	ma, ba, be	dk hairy	Nov. '96	n	ne	Thuya	n	n	n	20	1.5	47	40			
9	22-Jan-97	1856	01-13-57	36-42-51	lower Kabete	RB2	322	f	15417	UM3	ma, sp, ca	dk hairy	dk	n	ne	Kinuthia	n	n	n	5	1.5	30	25			
10	22-Jan-97	1841	01-13-54	36-43-08	lower Kabete	RB2	700	f	14338	UM3	ma, man, ba, ca, lo	dk hairy	dk	n	ne	Njuguna	n	n	n	recent	1.5	40	31			
11	22-Jan-97	1894	01-14-01	36-43-19	lower Kabete	RB2	2812	s	13373	UM3	av	dk hairy	Dec. '96	n	dk	Kiarie	y	y	n	7+	0.5	83	60	60	10	16.7
12	22-Jan-97	1926	01-13-34	36-42-43	lower Kabete	RB2	624	s	15358	UM3	ma, ca	dk mixed	Nov. '96	n	ne	Njonjo	n	n	n	5	0.5	53	44			
13	22-Jan-97	1899	01-13-28	36-43-30	lower Kabete	RB2	612	f	17696	UM3	co	dk hairy	Oct. '96	n	dk	Gatundu	y	y	y	10+	1.5	69	66	66	27	40.9
14	30-Jan-97	1798	01-12-31	36-44-33	Karura	RB2	190	f	15196	UM3	ma	dk smooth	dk	n	dk	Wina Furahi school	n	n	n	dk	0.5	28	23			
15	30-Jan-97	1835	01-12-37	36-44-49	Karura	RB2	682	f	16553	UM3	ma, ba	mixed	Aug. '96	n	ne	Kamau	y	y	y	3	1.75	38	34	34	2	5.9
16	30-Jan-97	1912	01-12-42	36-45-08	Karura	RB2	3158	f	15629	UM3	ma	dk smooth	Dec. '96	n	within farm	Muuru	n	n	n	2	0.5	58	49			
17	30-Jan-97	1850	01-12-48	36-45-07	Karura	RB2	147	f	18500	UM3	co	mixed	Nov. '96	n	within farm	Muuru	y	y	y	1	0.75	37	37	37	5	13.5
18	30-Jan-97	1847	01-11-55	36-43-34	Karura	RB2	1021	f	15170	UM3	co	dk	Aug. '96	n	Istock off Kiambu	Kamau	y	y	n	1	1.5	50	41	41	3	7.3
19	30-Jan-97	1853	01-11-35	36-43-39	Karura	RB2	2055	f	17815	UM3	napier	dk	Dec. '96	n	ne	Kang'ee	n	n	n	1	0.5	54	52			
20	11-Feb-97	1937	01-11-25	36-43-43	Karura	RB2	574	s	14147	UM3	none	dk	dk	n	dk	dk-plot on verge	n	n	n/a	dk	0.5	34	26			
21	11-Feb-97	1830	01-11-11	36-43-28	Karura	RB2	682	f	16102	UM3	ma, ba	dk	Jan. '97	n	dk	Mugai	n	n	n	infill	0.5	54	47			
22	11-Feb-97	1862	01-11-01	36-43-15	Karura	RB2	825	s	16444	UM3	co,ba	mixed	Dec. '96	n	brother	Njoroge	n	n	n	dk	1.5	54	48			
23	11-Feb-97	1953	01-11-07	36-43-00	Karura	RB2	181	f	18500	UM3	av, ma, ba	dk	Jan. '97	n	ne	Wanyuhi	y	y	y	4	1.5	22	22	22	1	4.5
24	11-Feb-97	1840	01-11-14	36-43-15	Karura	RB2	914	f	17267	UM3	ma, ba	smooth	Dec. '96	n	ne	Kihara	n	n	n	1	0.5	45	42			
25	11-Feb-97	1892	01-11-23	36-43-28	Karura	RB2	196	s	18500	UM3	ba, fl, gr	dk	Nov. '96	n	within farm	Thuku	n	n	n	4	0.75	22	22			
26	11-Feb-97	1817	01-11-37	36-43-44	Karura	RB2	439	s	16444	UM3	ma, gr	short	Jan. '97	n	dk	not present	y	y	n/a	dk	0.5	45	40	40	11	27.5
27	20-Feb-97	2650	00-54-52	36-37-11	Kinale	RB2	981	s	14800	UH1	pe, ca	dk smooth (bana)	not cut	n	ne	Gickeca	n	n	n	0.7	0.5	55	44			
28	20-Feb-97	2595	00-54-27	36-37-55	Kinale	UP1	716	f	14311	UH1	ma, ca	dk mixed (bana)	Dec. '96	n	ne	Muiruro	n	n	n	2	0.75	53	41			
29	20-Feb-97	2585	00-54-04	36-37-55	Kinale	UP1	na	s		UH1	ca	bana	Dec. '96	n	ne	Kinale h. school	n	n	n	2	0.5	na	na			
30	20-Feb-97	2619	00-54-03	36-37-31	Kinale	UP1	66	s	18500	UH1	po, ca, pe, rh	bana	Nov. '96	n	ne	Ngure	n	n	n	6	0.75	66	66			
31	20-Feb-97	2526	00-54-04	36-37-38	Kinale	UP1	131	s	15725	UH1	ca	bana	Jan. '97	n	ne	Njuguna	n	n	n	4	0.3	20	17			
32	20-Feb-97	2603	00-57-11	36-37-21	Magina	LP1	403	s	17224	UH2	napier	mixed bana	Jan. '97	n	ne	Moses	n	n	n	7	0.5	58	54			
33	20-Feb-97	2575	00-57-05	36-37-12	Magina	LP1	168	s	18060	UH2	po, pe, ma	mixed bana	Jan. '97	n	ne	Kiarie	n	n	n	22	0.4	42	41			
34	20-Feb-97	2609	00-57-00	36-37-28	Magina	LP1	68	s	16324	UH1	ca, fallow	bana	Dec. '96	n	ne	Waboye	n	n	n	2	0.5	17	15			
35	27-Feb-97	2262	01-00-07	36-37-06	Escarpment	RB3	192	f	17729	LH2	fa	dk bana	Jan. '97	n	dk	Mbugua	y	y	3 yr	15	0.3	96	92	92	37	40.2
36	27-Feb-97	2280	01-00-07	36-36-54	Escarpment	RB3	24	f	17713	LH2	fa, po	bana	Jan. '97	n	ne	Kamau	n	n	n	2	0.3	47	45			
37	27-Feb-97	2361	01-05-04	36-38-02	Bibirioni	RB3	239	s	14536	UH2	ma	dk bana	Dec. '96	n	ne	Ndungu	n	n	n	5	0.75	28	22			
38	27-Feb-97	2377	01-05-05	36-38-26	Bibirioni	RB3	41	f	17146	UH2	fa, fruit trees	bana	Jan. '97	n	ne	Gathunga	y	y	2 yr	5	0.5	41	38	38	3	7.9
39	27-Feb-97	2356	01-05-22	36-38-29	Bibirioni	RB3	746	f	12488	UH2	ma, ca, na	bana	Jan. '97	n	ne	Njeri	y	y	1 yr	2	0.5	40	27	27	3	11.1
40	27-Feb-97	2371	01-05-19	36-38-14	Bibirioni	RB3	330	f	17883	UH2	ca, fruit trees	dk bana	Oct. '96	n	ne	Njeri	y	y	y	8	1	30	29	29	4	13.8
41	27-Feb-97	2418	01-05-40	36-38-04	Bibirioni	RB3	2379	f	13942	UH2	ma, ca	dk bana	Jan. '97	n	ne	Kiratu	y	y	1 year	5	0.2	69	52	52	2	3.8
42	27-Feb-97	2341	01-05-45	36-38-28	Bibirioni	RB3	173	s	11261	UH2	po, ca, or	mixed	Jan. '97	n	ne	Kiratu	n	n	n	5	0.3	23	14			
43	27-Feb-97	2392	01-05-56	36-38-31	Bibirioni	RB3	102	f	17775	UH2	ma, fa, fruit trees	bana	Jan. '97	n	ne	Njeri	y	y	3 yr	10	0.6	51	49	49	7	14.3
44	11-Mar-97	2274	01-04-51	36-37-44	Bibirioni	RB3	400	f	16188	UH2	pe. ca	bana mixed	Nov. '96	n	ne	Kamau	y	y	3 yr	12	0.5	40	35	35	2	5.7
45	11-Mar-97	2361	01-05-00	36-37-23	Bibirioni	RB3	75	s	17760	UH2	ma, ca, po	bana dk	Jan. '97	n	ne	Kimotho	y	y	no	5	0.5	75	72	72	1	1.4
46	11-Mar-97	2324	01-04-45	36-37-10	Bibirioni	RB3	676	s	15500	UH2	ca, po	bana dk	Nov. '96	n	ne	Gikonyo	y	y	2 yr	3	0.4	37	31	31	1	3.2
47	11-Mar-97	2316	01-04-21	36-37-06	Bibirioni	RB3	1685	f	16596	UH2	pe	bana dk	Dec. '96	n	ne	Ndungu	y	y	no	3	0.75	68	61	61	1	1.6
48	11-Mar-97	2313	01-03-53	36-37-13	Bibirioni	RB3	754	s	10511	UH2	pe, po, ca	dk	Jan. '97	n	ne	Ngungu	y	y	3 yr	1	0.75	44	25	25	3	12.0
49	13-Mar-97	2168	01-08-58	36-36-09	Nderu	RB3	2590	f	9250	LH3	na, fa	bana dk	Feb. '97	n	ne	Murithi Sec. Sch.	n	y	2 yr	1	0.2	72	36			
50	13-Mar-97	2152	01-09-38	36-35-38	Nderu	RB3	283	f	13875	LH3	ca, ba	bana dk	Feb. '97	n	MOALDM	Wambui	n	y	1 yr	2	0.2	28	21			

APPENDIX 5 (cont.)

51	13-Mar-97	2192	01-09-59	36-35-45	Nderu	RB3	3114	f	9620	LH3	fa	bana dk	Feb. '97	n	ne	Thuo	n	y	5 yr	3	0.3	100	52			
52	13-Mar-97	2147	01-10-31	36-35-53	Nderu	RB3	200	f	17760	LH3	na, fa	bana dk	Feb. '97	n	ne	Kimani	n	y	1 yr	3	0.2	100	96			
53	13-Mar-97	2156	01-10-40	36-36-03	Nderu	RB3	525	s	15136	LH3	py, fa	bana dk	Jan. '97	n	ne	Githica	n	y	1 yr	1	0.4	33	27			
54	20-Mar-97	1751	01-03-06	36-50-31	Kiratina	RB3	96	f	17729	UM2	co, road	dk bana	Feb. '97	n	ne	Mbambi	n	n	1 yr	0.25	0.4	48	46			
55	20-Mar-97	1753	01-02-53	36-50-20	Kiratina	RB2	340	s	17202	UM2	ma, road	dk bana	Feb. '97	n	ne	Kamau	n	n	no	3	0.5	57	53			
56	20-Mar-97	1785	01-02-35	36-50-02	Kiratina	RB2	443	f	17267	UM2	ba	dk mixed	Feb. '97	n	ne	Wairimu	n	n	no	6	0.75	30	28			
57	20-Mar-97	1832	01-02-22	36-49-44	Kiratina	RB2	71	f	18500	UM3	ma, ba	dk bana	Jan. '97	n	ne	Nganga	n	n	no	3	1	24	24			
58	20-Mar-97	1863	01-02-12	36-49-19	Kiratina	RB2	157	s	16444	UM3	ma, ba	dk bana	Dec. '96	n	ne	Nganga	n	n	no	3	0.75	18	16			
59	20-Mar-97	1806	01-02-10	36-48-56	Kiratina	RB2	151	f	16553	UM3	ma, co, ba	dk bana	Dec. '96	n	ne	Kungu	n	n	1 yr	4	0.75	19	17			
60	26-Mar-97	1915	01-04-01	36-47-12	Kanjai	RB2	570	f	16235	UM1	ba, na	dk bana	Jan. '97	n	ne	Wanyoke	y	y	1 yr	1	0.75	49	43	43	3	7.0
61	26-Mar-97	1910	01-04-04	36-47-36	Kanjai	RB2	639	s	16349	UM1	ba, co, na	dk bana	Mar. '97	n	ne	Nganga	y	y	this year	2	0.5	43	38	38	11	28.9
62	26-Mar-97	1889	01-03-54	36-48-09	Kanjai	RB2	1351	f	17077	UM1	na, fa, ba, ca	dk mixed	Feb. '97	n	ne	Wataku	n	y	this year	5	0.5	52	48			
63	26-Mar-97	1816	01-03-57	36-47-57	Kanjai	RB2	196	s	17659	UM1	ba, ma	dk bana	Feb. '97	n	ne	Njambi	n	n	no	2	0.5	22	21			
64	26-Mar-97	1935	01-04-03	36-48-13	Kimathi	RB2	198	s	18500	UM1	ba, na	dk bana	Jan. '97	n	ne	Njoroge	n	n	no	5	0.7	20	20			
65	26-Mar-97	1791	01-04-21	36-48-25	Kimathi	RB2	539	s	16958	UM2	fa	dk mixed	Feb. '97	n	ne	Mbugua	n	y	this year	4	0.3	36	33			
66	26-Mar-97	1837	01-04-36	36-48-35	Kimathi	RB2	63	s	18500	UM2	ba	dk mixed	Jan. '97	n	ne	Mburu	n	n	no	6	0.5	21	21			
67	26-Mar-97	1785	01-04-48	36-48-54	Kimathi	RB2	578	s	15779	UM2	ba, fa, na	dk bana	Feb. '97	n	ne	Kimani	n	n	3 yr	1	0.3	34	29			
68	26-Mar-97	1738	01-05-11	36-49-20	Kimathi	RB2	247	f	17020	UM2	na, ba	dk bana	Dec. '96	n	ne	Mburu	n	n	no	1	0.75	25	23			
69	30-Apr-97	2039	01-06-02	36-43-59	Githiga	RB2	178	s	18500	LH1	co, road	dk bana	Jan. '97	n	within	Wakana	y	y	no	4	1	36	36	36	1	2.8
70	30-Apr-97	2059	01-05-50	36-43-53	Githiga	RB2	1329	f	16365	LH1	ca, ma, na	dk mixed	Mar. '97	n	ne	Njuguna	y	y	no	1	1	52	46	46	4	8.7
71	30-Apr-97	2195	01-05-32	36-44-05	Githiga	RB2	112	s	18500	LH1	po, ca	dk mixed	Mar. '97	n	ne	Njoki	y	y	1 yr	5	0.75	56	56	56	11	19.6
72	30-Apr-97	2105	01-05-25	36-44-15	Githiga	RB2	389	f	18500	LH1	na, ma	dk bana	Mar. '97	n	ne	Nganga	y	y	1 yr	1	2	37	37	37	2	5.4
73	30-Apr-97	2091	01-05-29	36-44-32	Githiga	RB2	105	s	17443	LH1	ma, ca	dk mixed	Mar. '97	n	ne	Wangoi	n	y	1 yr	2	0.5	35	33			
74	30-Apr-97	2188	01-04-42	36-42-27	Gitiha	RB2	781	f	16650	LH1	na, be, ca, po	dk bana	Dec. '96	n	within	Wamiytha	y	y	this year	2	0.5	40	36	36	7	19.4
75	30-Apr-97	2286	01-04-47	36-42-09	Gitiha	RB2	52	f	18500	LH1	ba, ma, po, spinach	dk	Aug. '96	n	ne	Gichuki	y	y	4 yr	2	2	26	26	26	6	23.1
76	30-Apr-97	2374	01-04-42	36-41-49	Gitiha	RB2	625	s	18013	LH1	ca, pas, fl	ordinary	not yet cut	n	within	Mbugua	y	y	no	0.5	1	38	37			
77	07-May-97	1977	01-06-02	36-48-01	Ikinu	RB2	281	f	17729	UM2	ma, po, ba	bana broadleaf	Feb. '97	n	Kiambaa	Wangeci	n	n	no	3	1	24	23			
78	07-May-97	1857	01-06-10	36-48-23	Ikinu	RB2	129	f	18070	UM2	ba, ma, road	dk bana	Apr. '97	n	ne	Mukora	n	y	this year	4	0.75	43	42			
79	07-May-97	1840	01-06-21	36-48-48	Ikinu	RB2	416	f	18500	UM2	ba, ma, na	dk mixed	Jan. '97	n	ne	Njambi	n	n	no	3	1	29	29			
80	07-May-97	1784	01-06-34	36-49-11	Ikinu	RB2	521	f	18500	UM2	ma, be	dk smooth	Dec. '96	n	ne	Wangui	n	n	2 yr	7	1.5	58	58			
81	07-May-97	1891	01-06-02	36-47-27	Ikinu	RB2	113	f	18500	UM2	co, ba	dk smooth	Apr. '97	n	within	Wancegi	n	n	1 yr	4	1.75	19	19			
82	07-May-97	1817	01-06-00	36-47-11	Ikinu	RB2	124	f	18500	UM2	co, ba, ma	dk smooth	Jan. '97	n	ne	Mbele	n	n	no	4	1.5	62	62			
83	07-May-97	1951	01-05-57	36-46-53	Ikinu	RB2	69	f	18500	UM2	ba, ma, be	dk smooth	Feb. '97	n	ne	Waewa	n	n	1 yr	1	1.75	13	13			
84	07-May-97	1987	01-05-53	36-46-34	Ikinu	RB2	690	f	18500	UM2	co, po, ma, na	dk mixed	Jan. '97	n	within	Mbugua	y	y	1 yr	7	1.75	44	44	44	3	6.8
85	07-May-97	1876	01-05-55	36-46-14	Ikinu	RB2	835	f	16721	UM1	ma, man, be	dk smooth	Jan. '97	n	within	Ndegwa	n	n	no	1	1.5	52	47			
86	14-May-97	1874	00-54-13	36-51-15	Gituamba	RB1	424	s	17922	LH1	na	dk bana rough	Mar. '97	n	ne	Mwangi	n	n	no	5	0.75	32	31			
87	14-May-97	1914	00-54-06	36-50-59	Gituamba	RB1	274	s	17827	LH1	ma, road	dk mixed	not yet cut	n	ne	Ngawe	n	n	no	0.5	1	55	53			
88	14-May-97	1860	01-00-50	36-50-29	Kiamworia	RB2	48	s	16958	UM1	ma, ba	dk rough bana	Jan. '97	n	ne	Wairimu	n	n	no	1	0.5	24	22			
89	14-May-97	1885	01-00-39	36-50-19	Kiamworia	RB2	27	f	18500	UM1	ma, ba, be, po	dk rough	Feb. '97	n	ne	Mwangi	n	n	no	1	0.5	27	27			
90	14-May-97	1819	01-00-35	36-50-01	Kiamworia	RB2	58	f	17862	UM1	ma, ba, be, po	dk rough	Feb. '97	n	ne	Njoro	n	n	1 yr	3	2	29	28			
91	14-May-97	1640	01-02-26	36-53-29	Gathage	RB2	99	f	18500	UM2	fa	dk smooth, narrow	Jan. '97	n	ne	Chege	n	n	no	1	0.75	33	33			
92	14-May-97	1710	01-02-38	36-53-40	Gathage	RB2	157	s	18500	UM2	ma, be, ba, na, castor	dk hairy bana	Mar. '97	n	ne	Njuguna	n	n	1 yr	4	0.75	20	20			
93	14-May-97	1686	01-02-16	36-53-02	Gathage	RB2	76	s	18500	UM2	co, road	dk rough bana	Feb. '97	n	ne	Njeri	n	n	no	1.5	0.5	14	14			
94	14-May-97	1770	01-02-03	36-52-48	Gathage	RB2	168	s	17760	UM2	co, po, ka, road	dk hairy bana	Feb. '97	n	ne	Chege	n	n	1 yr	5	0.5	25	24			
95	29-May-97	1433	01-09-53	37-04-49	Kalimoni	LB8	28	f	18500	UM5	ma, be	dk hairy	dk	n	ne	Kamau	n	n	no	4	1	14	14			
96	29-May-97	1454	01-09-50	37-04-33	Kalimoni	LB8	185	f	18500	UM5	ma	dk smooth	Apr. '97	n	ne	Munene	n	n	no	1	0.6	21	21			
97	29-May-97	1496	01-09-29	37-05-23	Kalimoni	LB8	220	f	18500	UM5	ma, be	dk mixed	Dec. '96	n	ne	Kagai	n	n	no	5	1.5	41	41			
98	29-May-97	1549	01-09-00	37-05-33	Kalimoni	LB8	44	f	14295	UM5	ma, ba, cas	dk mixed	dk	n	ne	Waweru	n	n			1	22	17			
99	29-May-97	1491	01-08-49	37-05-38	Kalimoni	LB8	44	f	16818	UM5	ma, be	dk mixed	Sept. '96	n	ne	Wainana	n	n	no	1	1	22	20			
100	29-May-97	1465	01-08-15	37-06-39	Kalimoni	VC	20	f	18500	UM5	ma, be	dk smooth	Nov. '96	n	ne	Waweru	n	n	no	3	2.5	10	10			
101	29-May-97	1487	01-08-14	37-07-40	Kalimoni	VC	99	s	18500	UM5	ba, ca	dk mixed	Mar. '97	n	ne	Ruhi	n	n	no	1	2.75	33	33			
102	29-May-97	1418	01-08-15	37-07-48	Kalimoni	VC	514	f	18115	UM5	ma, na	dk hairy	Mar. '97	n	ne	Kimani	n	n	no	4		48	47			
103	29-May-97	1383	01-08-14	37-08-25	Kalimoni	VC	2886	f	15654	UM5	ma	dk hairy	Apr. '97	n	ne	David	n	n	no	1	3	78	66			
104	29-May-97	1483	01-08-13	37-08-22	Kalimoni	VC	616	s	18038	UM5	ma, na	dk hairy	not cut	n	ne	Ndegwa	n	n	no	0.5	0.5	40	39			
105	29-May-97	1454	01-08-22	37-08-20	Kalimoni	VC	1023	f	6691	UM5	intercrop be	dk hairy	Apr. '97	n	ne	Gitau	n	n	no	2	0.3	47	17			
106	29-May-97	1430	01-08-28	37-08-21	Kalimoni	VC	92	f	16891	UM5	ma, be	dk hairy	Mar. '97	n	ne	Kanyai	n	n	no	4	2.5	46	42			
107	05-Jun-97	1549	01-01-11	37-00-38	Kariminu	RB3	60	f	18192	UM3	ma, ba, be	dk hairy	Apr. '97	n	ne	Muchiri	n	n	no	29	0.75	60	59			
108	05-Jun-97	1579	01-00-57	37-00-21	Kariminu	RB3	68	f	17678	UM3	ma, ba, be, pe	dk hairy	May '97	n	ne	Ngige	n	n	no	12	1	45	43			
109	05-Jun-97	1672	01-00-38	36-59-58	Kariminu	RB3	264	s	16891	UM3	co	dk hairy	May '97	n	ne	Kagore	n	n	no	3	0.3	23	21			

APPENDIX 6

An agro-ecological approach to the management of a smut disease caused by *Ustilago kamerunensis* on Napier grass, *Pennisetum purpureum,* in Kenya.

G FARRELL[1], S A SIMONS[2] and A M JULIAN[3]

[1]KARI/DFID NARP II, P O Box 14733, Nairobi, Kenya
[2]CAB International Africa Regional Centre, PO Box 76520, Nairobi, Kenya
[3]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK

Background and objectives

Napier grass (*Pennisetum purpureum* Rich. in Persoon) is the major source of fodder for zero-grazed livestock in Kenya. In the early 1990s a new disease of the grass was brought to the attention of the Ministry of Agriculture by farmers. The pathogen was identified as *Ustilago kamerunensis* H Sydow and Sydow [1]. It had been reported as a minor problem from other East African countries but has proved to be more severe and widespread in Kenya (affected plants produce a smutted inflorescence and biomass production is much reduced). This may reflect the presence of a more virulent strain, less resistant Napier grass clones or a greater use of the grass in Kenya. The grass has been heavily promoted for stall fed animals since the 1960s, with a consequent increase in the area under cultivation.

The overall objective of this investigation is to develop management strategies for Napier grass smut for smallholder farmers through an understanding of the pathogen's biology and ecology. The work reported here gives details of a disease survey of the crop and development of a predictive model for the incidence of Napier grass smut.

Materials and methods

A stratified two stage random sample of 109 Napier grass plots was done in 1997 in a high potential area (Kiambu District) of central Kenya. In each plot a diagonal transect was laid out. Parameters noted at 1m intervals were presence of smut and other pests and diseases. Questions to farmers elicited information on the age of the Napier crop, its height, the grass type, presence of smut in the field (even if not on the transect), smut history, source of Napier grass, date of last crop cut, slope of the plot, identity of adjacent crops and general comments on the health of the stand. The geographical coordinates of the plot was measured using the Global Positioning System. From the coordinates, the soil type, agro-ecological zone and administrative location of each plot were determined from maps. The agro-ecological parameters were subjected to an analysis of deviance to develop a model of proportion of smut.

Results and conclusions

Napier plants from nine agro-ecological zones and seven soil types were sampled. Of 3925 Napier plants examined, 171 (4.4%) had smut disease. The proportion of plots infected was 25%. No other major pests or diseases were found. Many farmers did not recognise that infected plants were diseased (they assumed that infected plants were a different species) and so the importance of the disease has probably been underestimated.

Napier plants were wholly smutted (all stems on a stool infected) or partially smutted, in that only some of the stems showed signs of disease. It seems that this pathogen is partially systemic. The fungus is able to initiate disease within a stem but not to infect other stems on the same stool ie infection is vertically systemic but not horizontally systemic. Experience suggests that removing diseased parts of a stool leads to disease remission within that stool, and could be recommended as a control measure. If plants can be partially smutted, it should be possible to relate the number of smutted stems to a loss value for biomass and hence develop a scoring system for disease severity.

Spatial pattern analysis revealed non-clustering of plants in 72% of the plots, suggesting that plant-to-plant disease transmission is of minor importance, and that diseased plants do not act as major foci of infection within a plot. This suggests that the infection behaviour of *U. kamerunensis* is analogous to that of soil borne pathogens.

The model of the proportion of smut was based on a quadratic function of altitude, as the only significant parameter. This is the first published smut disease correlative model that uses environmental factors. Underlying parameters that contribute to the altitude effect, such as temperature, rainfall or leaching of soil nutrients, have not been elucidated.

To develop management advice for farmers, future work will investigate sources of inoculum (spore dispersal and distribution of infected planting material),factors influencing the spread of the disease (such as soil amendments) and build on existing farmer practice in roguing diseased plants. It may be possible to recommend a fallow period if spore survival in soil is short. Longer term control measures, as part of a basket of options for farmers, could involve resistant clones, alternative fodders such as Guatemala grass (*Tripsacum fasciculatum*) or fungicides. However, with such a traditionally low input crop, farmers require methods of influencing the disease that are free or cheap.

References

1. Mordue J E M (1993) International Mycological Institute Biosystematics Services Report H380/93/YE1.

APPENDIX 7

KARI

DFID

A new disease of Napier grass
Management advice for farmers

A serious disease of Napier grass, caused by a smut fungus, has recently been reported in Kenya. The fungus is spread by spores and infected planting material. Infected stems are smaller in size and produce much less leaf than healthy plants, and so there is less plant material to feed cattle. Diseased plants develop short flowering stems in which the flower head is a mass of black spores. Infected plants usually flower early. There is no evidence that the fungus affects cows fed on infected plants. Use of fungicides is not recommended because the fungus grows inside the plant. The disease is found in the higher areas of Kiambu, Thika, Nyeri, Othaya and Kirinyaga Districts.

↑ farmer removes a smutted plant

← farmer holding a smutted stem

How to control the disease

1. Farmers should inspect their crop regularly and remove diseased plants as soon as they are found. There is no point leaving them because they will yield very little and just spread the disease. Diseased plants should be burned and not fed to livestock.

2. Obtain planting material only from areas which are free of the disease and select disease-free plants for new plots and for filling gaps.

3. Improve the health of the Napier crop by applying manure or fertilizer (4 bags per year per acre NPK 20-10-10, 2 bags in middle of long rains and 2 bags at beginning of short rains). Make sure the crop is weed free.

4. Consider growing alternative fodders, such as Guatemala grass, Giant Panicum or Giant Setaria.

KARI is searching for sources of resistance to this smut disease. Any resistant material would be released to farmers for testing.

G Farrell
KARI/DFID National Agricultural Research Project II
NARL, PO Box 14733, Nairobi tel/fax 443956 e-mail cpp@arcc.or.ke
NARC Muguga, PO Box 30148, Nairobi tel 0154 32880 fax 0154 32348
UK Department for International Development