SB
197
.R65

Project title:	**THE ROLE OF ENDOPHYTES IN TROPICAL FORAGES**
Executing Agency:	**Centro Internacional de Agricultura Tropical (CIAT)**
Collaborating Partner:	**Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF)**

CIAT
UNIDAD DE INFORMACION Y DOCUMENTACION
13 DIC. 2005
222020
CIAT
COLECCION HISTORICA

September 20, 1994

CIAT/GOVERNMENT OF JAPAN COLLABORATIVE RESEARCH PROJECT

Project Title: **The Role of Endophytes in Tropical Forages**

Donor Contribution 1996 :US$200,000

September 20, 1994

PROJECT DESCRIPTION

I. BACKGROUND

The word endophyte refers to fungi which reside entirely within plant tissues. Endophytic fungi are reported in grasses, shrubs, and evergreen trees, and are most likely to be found in most host plants. Many grasses harbor clavicipitaceous endophytes within their leaves, stems, or seeds. It has been demonstrated that some of these endophytic fungi confer beneficial effects on infected plant hosts. In some grasses, endophytes provide increased resistance to some insects, plant pathogens, and increased tolerance to drought. In addition to resisting attacks by insects, endophyte-infected plants often exhibit more vigorous growth. Endophyte-infected plants may also contain toxic alkaloids which can cause toxicity in animals which graze them.

The presence and role of fungal endophytes is well documented in temperate grasses such as *Lolium, Hordeum, Aegilops, Secale, Triticum, Avena, Festuca,* and *Bromus*. In temperate grasses, it is now common to ensure that seed of grasses carries beneficial endophytes. These positive effects have been reviewed recently (Bacon, 1994, Appendix I). Endophytes are used commercially to confer insect resistance in fescue in the United States and in most turf grasses in Europe and the United States.

The role of endophytes in tropical grasses, however, is largely unknown. Beneficial effects have not been established. But it is likely that some known cases of insect resistance can be attributed to endophytes. Detrimental effects have been attributed to endophytes but there is no proof of such effects.

Tropical grasses are widely sown in South America, the most common being different species of *Brachiaria,* of which there are approximately 50 million hectares. Differences in tolerance to insects has been observed between species and accessions. Also phytotoxicity problems have been observed in animals grazing *Brachiaria* species. It has been suggested that the problem may be due to an endophyte producing toxins, however, this has not been firmly established (Appendix II). Most previous investigations have been carried out by clinical veterinarians without the involvement of pathologists and forage agronomists.

There is also evidence of beneficial effects of endophytes on crop plants, e.g. preliminary research results from CIAT which demonstrated increased yields of cassava (Appendix III).

The use of naturally occurring biocontrol agents for disease and insect protection will counter the adverse environmental consequences of heavy pesticide use. The use of endophytes as a natural control agent has certain appeal and advantages. One of these is that endophytes can be transmitted through ovules and seeds of their plant hosts. This means that once the desirable endophyte is identified and characterized, plant inoculations need be done only once. Desirable cloned genes may also be introduced and maintained in these endophytes.

Results of strategic research on endophytes will have widespread application. The results are of particular significance to small farmers because of the low cost of applying the research results and savings in the reduced use of pesticides.

It is an appropriate time to introduce such research because mechanisms are currently being put in place for increased interaction between International Agricultural Research Centers. CIAT will participate in the new Systemwide Livestock Program coordinated by ILRI and involving CIAT, ICARDA, ICRISAT, ICRAF, IITA and IRRI. It will be easy to expand the activities to areas in Asia and Africa once procedures have been established at CIAT.

In summary, the identification and characterization of endophytes in tropical forages will enable us to document and identify them, determine their role in enhanced resistance of forages to insects, plant pathogens and drought tolerance, clarify their role in toxicity to cattle, and extend the techniques to the use of endophytes in other crops. There are good chances of success because of the advances already made in the knowledge and application of endophytes in temperate forages.

II. RESEARCH OBJECTIVES AND ACTIVITIES

The research objectives are:

(i) to determine the extent of endophyte occurrence, firstly, in tropical forages and, secondly, in other crops,

(ii) to obtain evidence of the possible role of endophytes in pest and disease management of forages and other crops,

(iii) to determine a possible relation between endophyte occurrence and toxicity of forages to livestock and

(iv) to demonstrate how this knowledge might be used to assist small farmers in more efficient and environmentally sustainable production.

The purpose, components,outputs and main activities of the Research Program are set out in Figure 1.

The strategy is to use the knowledge and experience that has been obtained in temperate areas and extend this to other tropical areas once the techniques have been adapted to tropical crops. Pathologists from Japan have expertise in the role of endophytes in forages and crops and would provide expertise and support to the Project.

Scientists from CIAT will provide support in pathology, in carrying out field trials with forages and crops and in the sheep feeding trials.

Figure 1: Work Breakdown Structure

Project Goal
To explore the use of bio-control agents in management of pests and diseases.

↓

Project Purpose
To survey and identify endophytes in tropical forages and determine their role in plant protection and effect on animal performance.

↓

	Component 1: Survey and identification of endophytes.	**Component 2**: Determine role in disease and pest control and agronomic performance.	**Component 3**: Determine role for endophytes in animal toxicity.	**Component 4**: Project Management.
Project Outputs	▸ Fungal endophytes of tropical grasses identified.	▸ Knowledge of specific effects of endophytes on pests and disease.	▸ Relation established between endophyte and toxicity.	▸ Well managed project and results disseminated widely.
Activities	▸ Survey occurrence in different ecoregions in Colombia. ▸ Survey occurrence in other countries in tropical America. ▸ Histopathological studies. ▸ Isolation and identification of endophytes. ▸ Extend survey to Asia and Africa.	▸ Plant inoculation studies in greenhouse. ▸ Examine role in disease control. ▸ Examine role in pest control in collaboration with entomologist. ▸ Field inoculation studies. ▸ Studies of agronomic performance of forage grasses. ▸ Studies of agronomic performance on cassava.	▸ Survey of areas where toxicity reported. ▸ Identification of endophytes and other fungi. ▸ Controlled feeding trials with sheep to simulate effect of specific endophytes on animal performance (Monitor intake, digestibility, visual symptoms, blood analysis). ▸ Post-mortem examination of dead animals for organ damage. ▸ Identification of toxic agent. ▸ Devise management strategy to minimize risk of toxicity.	▸ Appoint personnel. ▸ Monitor project and prepare annual report. ▸ Financial management and reporting. ▸ Liaison with different laboratories working on endophytes. ▸ Publish research reports. ▸ Hold international conference. ▸ Final report.

III. DURATION AND SCHEDULING

The Project will operate for 5 years. The sequence of activities is set out in Figure 2. Progress will be reviewed annually and reported by 30 November each year. This will allow for changes in the research schedule and proposals for small modifications in the budget.

IV. EXPECTED OUTPUTS

Fungal endophytes of tropical grasses and cassava identified

Beneficial endophytes identified for pest and disease control

Beneficial effects of endophytes on yield of forages and cassava demonstrated

Possibilities for commercial use of endophytes in disease and pest control established

Determined if endophytes are responsible for occasional outbreaks of toxicity in animals

Results of research published

International cooperation in endophyte research between International Agricultural Research Centers and National Research Centers established

International conference on endophyte research held

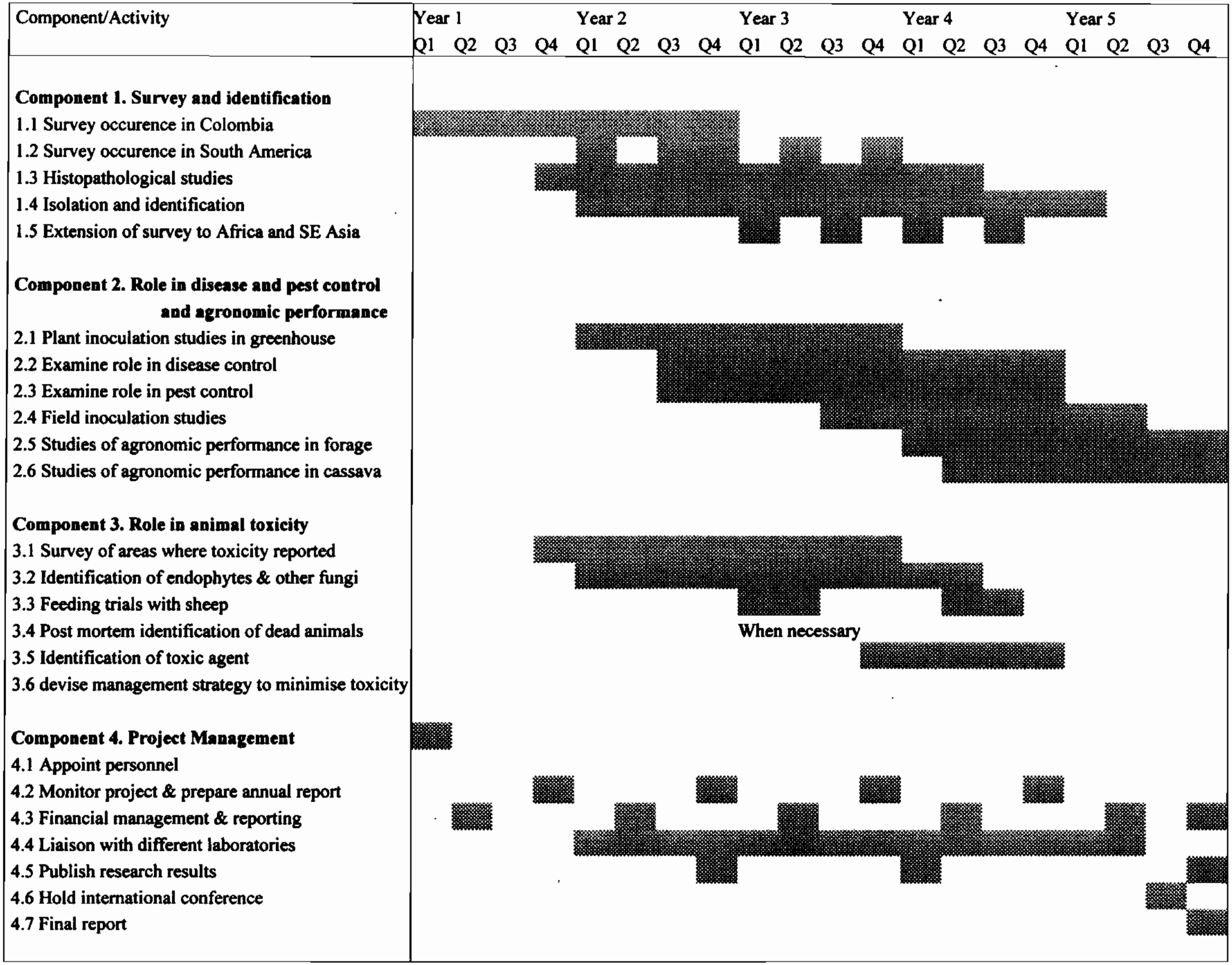
IV. INPUTS

The budget for the first year of operation is $200,000.

Inputs are required for:

Salary and on-costs of a senior scientist from Japan
Salary of an assistant, 2 technicians, part-time secretary and casual labor
Consultant support
Travel costs
Equipment purchase
Vehicle lease and maintenance
Laboratory materials
Office supplies and services
Indirect costs to CIAT (10%).

Figure 2. Project Activity Schedule - Endophyte Project



V. WORK PLAN FOR FIRST YEAR

After the signature of the Project Document by the Government of Japan and CIAT and the release of funds for the first year of operation by the Government of Japan, CIAT will appoint a Japanese scientist and local support personnel. The base of the Project will be at CIAT, Palmira, Colombia, and the scientist will conduct research there.

In the first year the following activities are planned:

Component 1
1.1 Survey of endophyte occurrence in Colombia
1.3 Histological studies of plants containing endophytes
1.4 Identification of endophytes

Component 2
To be commenced in Year 2

Component 3
1.1 Survey of areas where animal toxicity on *Brachiaria* has been reported
1.2 Commence identification of organisms causing toxicity

Component 4
4.1 Appointment of personnel
4.2 Project strategy discussed and annual progress report
4.3 Financial management of funds and report

VI. PRINCIPLES

1. The aim of the Project is to identify the extent of endophytic fungi in tropical forages and other crops and exploit such endophytes for forage and crop improvement.

2. The funds will be used exclusively for the implementation of the Project.

3. CIAT will be responsible for the execution of the Project. Activities under the Project will be subject to the availability of funds and as such obligations of CIAT are subject to receipt of funds.

4. Scientists and technical personnel will be appointed by CIAT in accordance with the terms and conditions of service applicable to CIAT personnel. The resident scientist who participates in this project will be a scientist belonging to the Ministry of Agriculture, Forestry and Fisheries, Government of Japan. This person will be treated in all respects as a CIAT staff member and will be directly responsible to CIAT for the conduct of research.

5. CIAT will establish appropriate records to account for all the funds received under the Project and to record all expenditures incurred in pursuit of the collaborative research effort, and will provide detailed financial reports to the Government of Japan within 60 days after the close of each fiscal period ending March 31.

6. CIAT will establish appropriate bank accounts to deposit all funds received from the Government of Japan and will maximize short term interest earnings of funds received in accordance with its policies on investment of Institute funds. Interest earned on undisturbed funds will be credited to the Project and used in consultation with the Government of Japan.

7. CIAT will provide the Government of Japan with a detailed annual progress report outlining the technical efforts and accomplishments of the Project by November 30 each year in time to allow any modification of the plans or budget for the following year.

8. Monitoring and supervising of the Project will be carried out jointly by CIAT and the Ministry of Agriculture, Forestry and Fisheries.

9. The results of the studies will be published by CIAT. Ownership of the materials and data resulting from the studies, including copyright, if appropriate, will be vested jointly in CIAT and the Government of Japan.

10. Any funds remaining on the completion of the Project will be reported to the Government of Japan and disposed of in accordance with the wishes of the Government of Japan.

APPENDIX I. REVIEW OF ENDOPHYTES

Source: Forage Quality, Evaluation, and Utilization. 1994. (Ed.) George C. Fahey, Jr. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

CHAPTER 8

FUNGAL ENDOPHYTES, OTHER FUNGI, AND THEIR METABOLITES AS EXTRINSIC FACTORS OF GRASS QUALITY

C. W. Bacon

INTRODUCTION

Considerable improvements in forage quality of grasses have been made within the past two decades. These improvements are consequences of genetic manipulations of grass cultivars and improved management strategies which guarantee that there is more than an adequate amount of grasses for grazing. These improvements are integral factors in the concept of forage quality. Nevertheless, animals grazed on certain properly managed grasses may still have reduced performance. Reduced animal performance is characterized by low gains, reproduction difficulties, low acceptability, and toxicity syndromes. The factors in forage grasses responsible for reduced animal performance are called antiquality components, and consist of a large diverse group of chemical constituents which may be divided into constitutive and extrinsic or exogenous antiquality components. Constitutive antiquality components are those that are produced directly by and are, therefore, inherent factors of grass species. Extrinsic antiquality components are those that are produced on a grass species but by another biological entity and are exogenous to the normal metabolism of that grass species.

This review is concerned with extrinsic antiquality factors of grasses produced by fungi which are commonly referred to as mycotoxins. These antiquality factors are biological, originating from a variety of fungi, some of which have been associated not only with animal performance problems but also human toxicities since ancient times. In this regard, species of *Claviceps* and their toxins (ergot alkaloids) are historically perhaps the first recorded groups of extrinsic biological antiquality factors reported in forages.

The primary focus of this chapter is to critically evaluate an expanding body of literature which centers around toxins associated with fungi which live within forage grasses as endophytes, resulting in an antiquality component of an otherwise desirable forage species. Evidence will be presented which establishes that most of the cultivated forage grasses are descendants of

---

USDA/ARS, Toxicology and Mycotoxin Research Unit, Richard B. Russell Agricultural Research Center, Athens, GA 30613.

either wild or ancestral species that are naturally infected with endophytes. Further, a considerable discussion pertaining to positive interactions of endophytes with grasses will be presented which should prove helpful in understanding the symbiotic operation of these organisms in natural systems. This latter discussion is warranted since the association of endophytic fungi with most of the major forage grasses is natural and ecologically significant and their removal from grasses may, in certain instances, result in poor forage productivity which might indirectly affect forage quality.

In this chapter, information from basic and applied sources has been assembled to illustrate unique characteristics of the endophyte-grass-livestock interaction, which has an evolutionary basis and is fundamental to the disastrous effects on a trophic interaction, referred to here as grazing. Because of space limitations, this review emphasizes antiquality factors and toxins related to fungal endophytes. However, there is another large group of fungi that also affects grass quality. These fungi, discussed in part two, are referred to here as mycotoxic nonendophytic fungi. Mycotoxic nonendophytic fungi include nonmutualistic saprophytes living on dead matter, endophytic latent fungi, and localized systemic pathogens of grasses. Nonendophytic fungi will be discussed very briefly since it is this group that has been recently reviewed extensively (see Smith and Henderson, 1991), and historically the numerous early studies of this group formed the basis for our understanding of toxic fungi in general.

PART I: GRASS ENDOPHYTES

Biological Concepts

Endophytic Species of Fungi

The endophytic fungi in this review include fungi of the tribe Balansiae (family Clavicipitaceae, class Ascomycetes) and their related anamorphs (Figure 1). The species within this tribe were initially delineated only on the nature and degree of association with ovaries and vegetative parts of grasses (Diehl, 1950). However, this classification system fails to recognize phylogenetic relationships among species, and does not account for any relationships of species with only an anamorphic state.

A recent consideration of the phylogeny of the clavicipitaceous fungi as expressed in Figure 1 indicates two major features of this family, one group parasitic on insects (Cordycepitoideae typified by species of *Cordyceps*), the other two groups parasitic on grasses (Bacon and Hill, 1994). This phylogeny is based on a series of studies which, in addition to the host-fungus relationships of Diehl (1950), also include fungal morphology, conidiation, biochemistry, and molecular biology (Sampson, 1933; Diehl, 1950; Luttrell, 1979; Luttrell and Bacon, 1977; Latch et al., 1984; Rykard et al., 1984; Rykard et al., 1985; Bacon et al., 1986; White, 1987; White, 1988).

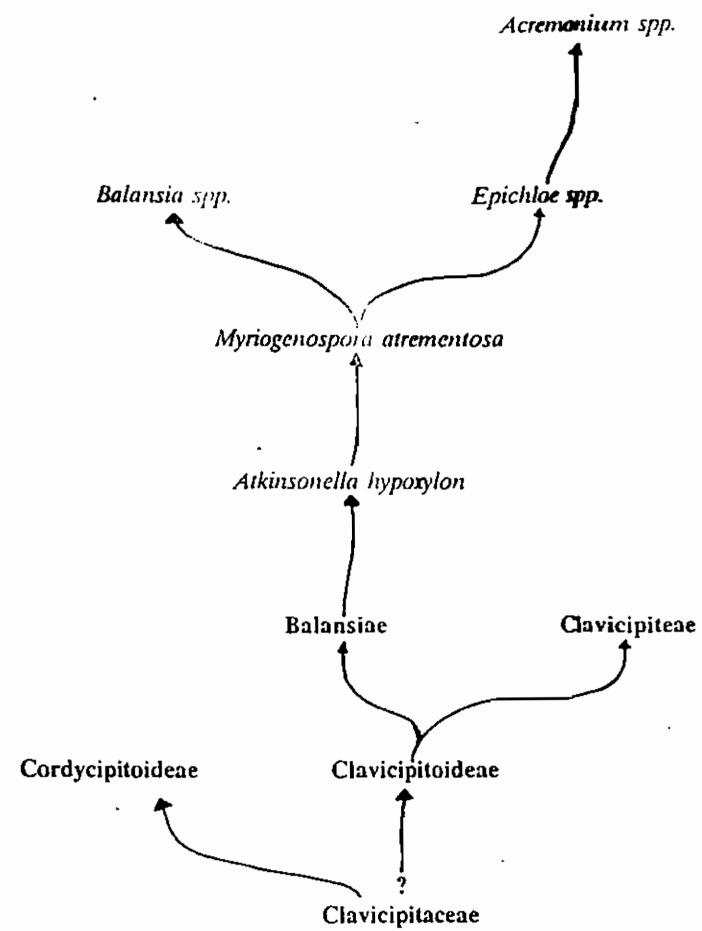


Figure. 1. Possible evolutionary lines of taxa among grass endophytes of the tribe Balansiae within the family Clavicipitaceae, subfamily Clavicipitoideae, beginning with an unknown (?) ancestor. The tribe Clavicipiteae and the subfamily Cordycipitoideae are illustrated for possible relationships within the family, although no cladistics is implied.

Considered from the above viewpoint, there are four major tendencies within the Clavicipitoideae: 1) a line indicating localized parasites of grass ovaries as shown by the *Claviceps* (Clavicipitaceae) and also characterized by having a stage independent of the grass, the sclerotium; 2) the change from a strictly ovarian pathogen and location, to also include the foliage parasitic habit (systemic) with varying degrees of the endophytic habit, and the loss of an independent stage from the grass (presently shown by the monotypic species *(Atkinsonella hypoxylon)*; 3) development of an endophytic habit but slightly pathogenic *(Myriogenospora atrementosa)* which culminates with species of *Balansia* characterized by a completely endophytic habit; and 4) the loss of sexuality, they are nonpathogenic, and are strictly seedborne and this group culminates in the development of the completely endophytic mutualistic habit (the *Acremonium* species). Thus, the *Acremonium* endophytes which are fungi associated with important forage grasses, also represent the most advanced group of grass endophytes.

This phylogeny serves to unify the Clavicipitaceae into one group but yet separates the endophytic fungi into two very broad categories: 1) those that produce external stromata (a mass of fungus hyphae which produces spores) on their grass hosts, and 2) those that do not and which, for the most part, are imperfect fungi only upon isolation in culture.

The first group include the fungi *Atkinsonella, Balansia, Epichloe,* and *Myriogenospora* which form external primary anamorphic stromata within or around stems, leaves, or florets of grasses from which either effuse, pulvinate, or stalked perithecial stromata arise and are, therefore, fungi of the first category. Most of these endophytic species are not associated with major forage grasses, but are associated with invasive grass species, particularly rangeland species (Diehl, 1950; White and Cole, 1986; White et al., 1990; White et al., 1992) (Table 1). Fungi of these genera are taxonomically related and have been placed in the tribe Balansiae of the family Clavicipitaceae (Diehl, 1950), and referred to here as the balansioid endophytes. Other essential distinguishing characteristics of species of this tribe are included in early studies (Luttrell and Bacon, 1977; Rykard et al., 1984; Bacon and De Battista, 1991).

The species in the second category include the genera *Acremonium, Gliocladium, Phialophora,* and *Pseudocercosporella*. Also included in this latter category are those endophytes that have been observed in a variety of annual ryegrasses (Latch et al., 1988; Nelson and Read, 1990). Most of these endophytic species are associated with important forage species and therefore represent the greatest economic concern to forage quality (Table 1). Since these species reproduce asexually by conidia, they belong to the form-class Deuteromycetes, and are, for the sake of convenience, separated from the balansioid endophytes and referred to here as the acremonioid endophytes. As presently defined, the genus *Acremonium* consists of *A. coenophialum* (Morgan-Jones and Gams, 1982) from two cultivars of tall fescue (*Festuca*

Table 1. Distribution of endophytes among the world's major forage grasses.*

Tribe/species	Common name	Endophyte genus
AGROSTIDEAE		
Agrostis alba, and other spp.	Bent grasses	*Acremonium*
Alopecurus pratensis	Meadow foxtail	*Acremonium***
Phelum pratense	Timothy	*
Sporobolus	Dropseed	*Balansia*
Stipa comata	Needle grass	*Acremonium*
AVENEAE		
Arrhenatherum elatium	Tall oatgrass	*Acremonium***
ANDROPOGONEAE		
Andropogon scoparium	Blue stem	*Balansia*
Sorghum vulgre, and other spp.	Forage sorghums	*Balansia*
CHLORIDEAE		
Bouteloua spp.	Grama grasses	*Balansia*
Chloris gayanam	Rhodesgrass	"
Cynodon dactylon	Bermudagrass	"
		"
FESTUCEAE		
Dactylis glomerata	Orchard grass	*Acremonium***
Elymus spp.	Wildryes	"
Eragrostis spp.	Lovegrasses	*Balansia*
Festuca spp.	Fescue grasses	*Acremonium*
Lolium spp.	Ryegrasses	"
Poa spp.	Bluegrasses	*Acremonium***
PANICEAE		
Axonopus offinis	Carpet grass	*Balansia spp.*
Panicum spp.	Panic grasses	*Balansia spp.*
Paspalum spp.	Dallisgrasses	"
Setaria italica	Foxtail millet	"

*The major forage grasses are compilations from Hoover et al., 1948; Bula et al., 1977; and Crowder, 1977. The distribution of endophytes is based on the work of Diehl, 1950; Kohlmeyer and Kohlmeyer, 1974; and White, 1987.

**Indicates that this endophytic species is *Epichloe typhina (A. typhinum)*.

arundinacea Schreb.) and from *Poa autumnalis* Muhl. ex Ell. (White and Bultman, 1987), *A. typhinum* from *F. rubra* L. (Morgan-Jones and Gams, 1982), *A. uncinatum* from *F. pratensis* L. (Gams et al., 1990), and *A. lolii* from *Lolium perenne* L. (Latch et al., 1988).

Since the *Acremonium* species are so agronomically important, much effort is being made to incorporate molecular data to clarify species relations. The essential premise is that *Epichloe typhina* which also produces an *Acremonium* state might be related to other *Acremonium* species. Ribosomal DNA was used to characterize nucleotide sequences among several species of endophytic fungi of grasses, including the *Acremonium* species (Schardl and Siegel, 1993). The results suggested a remarkable similarity of ribosomal DNA among all *Acremonium* organisms and that they were phylogenetically related to, and evolved from *E. typhina* (Schardl and Siegel, 1993). They also found that fungal mutualists did not necessarily coevolve with their grass hosts. In fact, as much dissimilarity occurred among *A. coenophialum* isolates from *F. arundinacea* as existed between *A. coenophialum* and *A. lolii* or *A. typhinum*. These findings raise doubts as to the true taxonomic derivation among species of *Acremonium* within sect. *Albo-lanosa*. Further, the ability of both the acremonioid and balansioid fungi to produce ergot alkaloids indicates at least a biochemical affinity among members of the Clavicipitaceae.

The Endophytic Habit

Symbiosis Endophytic fungi are those fungi which live their entire life cycle within the aerial portions of grasses and sedges by forming nonpathogenic and completely intercellular associations (Sampson, 1933; Diehl, 1950). The grass and fungus are symbiotically associated as a single ecological entity. Thus, in the discussions to follow, symbiotum (plural symbiota) or symbiotic will be used to refer to the traditional endophyte-infected grass terminology (Schardl et al., 1991) and nonsymbiotum to refer to noninfected grasses. The use of these terms will serve not only to emphasize the naturalness and importance of this association, but also to indicate potential problems which might occur if it is dismantled in attempts to remove the fungus as an antiquality factor.

The nature of the symbiosis varies among each of the major endophytic groups (Figure 1). Some symbioses are suggestive of incomplete, pathogenic relationships as shown by some of the *Balansia* species. The balansioid endophytes exist as free entities only very briefly during the spore stage and there is some question as to the significance of this spore as a dispersal unit. Unlike the balansioid endophytes, the acremonioid endophytes, as well as other forms of symbiotic associations (Ahmadjian and Paracer, 1986), do not live apart not even briefly, i.e., once a tall fescue or ryegrass symbiosis is established, the association is constant. The *Acremonium* symbioses are seed disseminated (Sampson, 1933), their association with the grass begins after germination of infected seed where upon the fungus infects developing seedlings, and symbiotic perennation is guaranteed by infecting the meristematic areas and all young vegetative organs except the roots.

Mutualism Regardless of the degree of the association, all endophytic fungi of grasses form biotrophic associations in which there is little or no destruction of either grass or fungus and as will be presented later, certain members exploit the biochemical properties of the other, indicating that in specific cases the relationship is mutualistic. Mutualisms are specific associations of two or more organisms that are characterized as interactions between individual organisms in which the genetic fitness of each participant is increased by the action of the other. This definition precisely describes the ecological nature of the symbiota and components of the mutualism are aptly referred to as plant or fungal mutualists.

Mutualisms are polymorphic in nature; thus, within a mutualistic population, there exists successful symbionts or true mutualists, unsuccessful symbionts whose fitness is not increased, and nonsymbiotic or noncohabitating individuals (Keeler, 1985). Most of the symbiotic grasses are of this first category and will be treated in this context. Examples of unsuccessful symbiotic grasses might reflect our inability to determine the survival value of specific traits under a precise environmental condition. It is rather doubtful that the third category of noncohabitating individuals exist, at least completely, since the fungi are only found in association with grasses. However, noninfected grasses of an infected species exist which may either occur naturally or they may be agricultural artifacts resulting from agronomic uses and practices, as for example, endophyte-free tall fescue (Bacon and Hill, 1994).

The tall fescue and perennial ryegrass symbiota are defensive mutualisms (Clay, 1988) because the overall competitive benefits (Hill et al., 1991a) are due to specific characteristics such as enhanced drought tolerance (Read and Camp, 1986; Arechavaleta et al., 1989), increased tillering and growth (Latch et al., 1985b; Hill et al., 1990), and increased resistance to herbivory from mammals and insects (Cheplick and Clay, 1988). The information available on *Balansia*-associated grasses suggest that they too are defensive mutualisms (Clay, 1984; Clay et al., 1985; Clay 1986).

The present data provide overwhelming evidence that interactions between the grass and fungus, as well as environment, increase the phenotypic variation among symbiotic plants (Clay, 1984; Kelley and Clay, 1987; Bradshaw, 1988; Hill et al., 1990). They also suggest that symbiotic plants do not have all mechanisms of fitness imparted upon them by the endophyte, but it is the sum of all the plants in the population which express the fitness characteristics (Bacon and Hill, 1994). Therefore, symbiotic populations of grasses are far more plastic and adaptable than nonsymbiotic populations. How fast symbiotic plants encroach upon nonsymbiotic plants in a mixed community will depend upon the types and severity of stresses imposed upon the plants in their environment. Undoubtedly, severe or repeated cattle grazing and other severe environmental stresses will eliminate nonsymbiotic grasses, while reduced grazing will have minimal effect on the competitiveness of a nonsymbiotic plant. Generally, the more ideal the growing conditions, the better the ability of the nonsymbiotic plant to compete with its symbiotic counterpart.

The endophytic habit is important not only from the standpoint that it is where the fungus resides but also because it is here that the fungus and (or) grass produce antiquality factors referred to as mycotoxins. Further, the nature of the endophytic habit is important because understanding will assist in formulating any possible control measures to remove the fungus. The endophytic habit was recognized as early as 1887 (De Bary, 1887) and described in the fungus *Epichloe typhina* (Pers. ex Fr.) Tul. The endophytic habit was subsequently established as a perennial feature of several grasses (Diehl, 1950), and this was followed by studies on the distribution of the endophyte within grasses (Sampson, 1933; Diehl, 1950). Most endophytes produce an external and transient sporulation structure (conidial stroma) upon which spores are produced which infect grasses.

Within an infected grass, the endophyte is intercellular and there is no evidence of any endophytic species producing mass destruction of host cells. However, this is not to imply that an endophyte does not 'create' a niche for itself within the grass. The intercellular spaces occupied by the fungus are not vacant spaces but spaces normally occupied by the middle lamellae of each cell. The endophyte apparently has the necessary enzymes to dissolve the middle lamellae as it grows between each layer of cells. There is no nutrient absorbing structure, typical of other parasitic fungi. Nutrient exchange takes place within this location, which consists of an external cellular plant matrix referred to as the apoplasm. The nutrients within the apoplasm may either be derived from the host due to normal cytoplasmic leakage and (or) from products derived from the digestion of the middle lamellae as the fungus grows between cells. This suggests that added nutrients will be available to the endophyte during periods of rapid plant growth, which would influence, and certainly correlates with, increased fungal growth and biochemical activities as evidenced by the periods of high ergot alkaloid content and herbivore toxicities (Belesky et al., 1987b; Belesky et al., 1988; Hill et al., 1990).

The extent of host colonization by an endophyte is endophyte-specific. Thus, species of *Balansia* and *A. hypoxylon* are found throughout all organs of the grass, except the roots. The species *Epichloe*, *Acremonium*, and *Myriogenospora* are found only within the sheath of leaves, meristematic areas, and inflorescence structures. Further, some endophytes appear to have a quantitative expression of hyphae within a host, e.g., the endophyte of the annual ryegrass while found in the sheath as discontinuous sparse fragments is found in much higher density within the seed (Latch et al., 1988; Nelson and Read, 1990). A single plant is usually not infected by more than one endophyte, but a grass species can serve as a host for several different endophytic species. However, double infection of a single grass plant by different genera has been reported, as in *Panicum anceps* infected by *M. atramentosa* and *B. henningsiana* (Luttrell and Bacon, 1977; Rykard, 1983). This may reflect occupation of different in situ locations. *Balansia* species are generalized and endophytic, whereas the infection by *M. atramentosa* is localized and systemic. Nevertheless, Latch et al., (1984) reported the co-occurrence of two endophytic fungi, *Gliocladium*-like and *Phialophora*-like, colonizing identical tissues along with *A. lolii* and *A. coenophialum* in plants of perennial ryegrass (*L. perenne*) and tall fescue, respectively.

Coevolution The reciprocal potential for genetic fitness derived from a mutualism serves as the driving force for the coevolution of endophytes and grasses. Plant-fungus associations appeared to have entered close relationships very early in their evolution. The Gramineae probably originated in the upper Cretaceous Period of the Mesozoic Era, much later than dicotyledonous plants. The fossil records of the first authentic species of grasses, fruits of *Stipa* (Cockerell, 1956) and *Phalaris* (Beetle, 1958), were obtained from the late Tertiary deposits, approximately 40 to 50 million years ago. The oldest fossil *Festuca* species was reported in the Miocene epoch of the Tertiary period (Thomasson, 1986). Thus, geologically speaking, we are dealing with a relatively young association which must have been initiated at least during the early Pliocene of the Cenozoic Era, approximately 25 million years ago.

To understand the need for a mutualistic relationship to develop between grasses and fungal endophytes, a complete understanding of the systematics and evolution of both the endophyte and species, their biochemical relatedness and requirements must be known. For more detailed discussions of this evolutionary aspect, as well as a model for fitness and other salient but theoretical evolutionary events within these mutualisms, the reader is referred to earlier reviews (White, 1988; Bacon and Hill, 1994). Briefly, the major impetus considered responsible for extending the adaptability of symbiotic grasses are the variety of secondary metabolites, most of which are produced by the fungus within the association.

Fungi are rated as highly adaptable because they have biochemical abilities to utilize a wide variety of substrates and produce precursors from intermediary metabolism for use in primary and secondary metabolism. On the other hand, grasses are one of the few groups of plants that lack the ability to produce excessive secondary metabolites (Zahner et al., 1983). In theory cohabitation and the establishment of a completely compatible association, although gradual, were based partially on the need for secondary metabolites that were lacking in the grass but were contributed by the fungus. The evolutionary events which resulted in the cohabitation of grasses with this group of fungi will probably remain unknown. It is clear, however, that the clavicipitaceous fungal mutualists meet the criterion that Law (1985) proposed for mutualism: 1) the inhabitants are genetically similar while their hosts are genetically diverse, 2) the inhabitants rarely or never undergo sexual reproduction, and 3) the inhabitants lack strong specificity to a particular host species.

Distribution of Endophytes Among Grasses

Endophytic fungi have been reported to infect grass species belonging to six subfamilies of the Gramineae as delineated by Gould and Shaw (1983) and several of these are considered important forage grasses throughout the temperate and tropical grazing zones of the world (Hoover et al., 1948; Bula et al., 1977; Crowder, 1977). Of the North American species of grasses, this represents a total of 232 associations (Bacon and De Battista, 1991). Thus, only 15% of all the New World's grass species are hosts for endophytes but whose distribution ranges from temperate to tropical zones. The distribution of symbiotic grasses appears to be limited to host habitat and climatic conditions. However, several grass species with widely different geographic distribution are infected with the same fungus at each end of their geographic extremes (Diehl, 1950).

Host-specific endophytes have not been demonstrated, although no comprehensive study of this phenomenon has been attempted. In natural grass communities, several host species may be found growing sympatrically but it is usually observed that only one species is infected by a particular fungal endophyte, suggesting that there is a compatibility factor. Experimental infections of grasses and sedges with *Atkinsonella hypoxylon* and *B. cyperi* established that these two fungi were broadly cross-compatible within a specific host population, but *B. cyperi* was less host-specific than *A. hypoxylon* (Leuchtmann and Clay, 1988).

As indicated above, the Balansioid endophytes are probably related to the *Acremonium* endophytes, so their distribution among the grasses will be discussed together (Table 1). Endophytes of this type are found associated with more grass species than any other endophyte (Latch et al., 1984; Williams et al., 1984; Halisky et al., 1985; White and Cole, 1985; White and Cole, 1986; Morgan-Jones et al., 1990; White et al., 1992). However, the distribution of this species within the subfamilies of the Gramineae is very narrow as 90% of the *E. typhina* is associated with the Festucoideae, mainly the Festuca species and other cool season grasses. This percentage includes the related *Acremonium* anamorphs. Both *E. typhina* and *Acremonium* sp. are the endophytes associated with major forage grasses. On the other hand, *Balansia* species are primarily associated with over 102 warm season grass and sedge species from all subfamilies except Bambusoideae. This distribution appears to favor two subfamilies since 97% of the *Balansia*-associated grasses include species of the Panicoideae (64%) and Eragrostoideae (33%) (Bacon and De Battista, 1991).

Endophytic fungi are found in approximately 15% of the North and South American species of grasses, many of which are used as forages (Bacon and De Battista, 1991). However, this percentage is probably much higher as it does not reflect a wide scale sampling of all the forage grass species for endophytes. The *Balansia*-associated grasses are not necessarily major forage species; they are, however, major North American rangeland species (Table 1). *Balansia epichloe* is the most cosmopolitan of the *Balansia* species, its

host range varies from cool-season grasses of the Festucoideae to warm-season grasses of the Panicoideae, Eragrostoideae and Arundinoideae. Other *Balansia* species have been reported from only one or two grass tribes or species. Thus, *B. ambiens*, *B. oryzae*, and *B. pallida* are associated only with grasses of the tribe Oryzeae, while other species of *Balansia* are found infecting only panicoid or eragrostoid grass species. Two species, *B. aristidae* and *B. hemicrypta*, are restricted to the grass genus *Aristida*, while *Balansia oryzae* is one of two species found in association with rice, which is the only known association of an endophyte with a forage crop used for human food (Mohanty, 1964). Two minor genera, *Atkinsonella* and *Myriogenospora*, are even more restrictive in their associations with grasses (Diehl, 1950). Nevertheless, *M. atrementosa* is associated with the Paspalum species which are used as forage grasses. *Atkinsonella hypoxylon*, *B. pilulaeformis* and *B. cyperi* are associated with a variety of native grasses and nongraminaceous hosts that are found throughout the major grasslands of the temperate and cooler tropical regions of the world.

Positive Benefits Derived from Symbiotic Grass

Natural populations of symbiotic grasses persist longer and compete better than nonsymbiotic populations (Clay, 1986; Clay, 1987; Prestidge et al., 1982; West et al., 1989). However, as discussed earlier the components of improved fitness of symbiotic populations are multifaceted and difficult to access. Most research on symbiotic grasses has emphasized variation of fitness at the population level, with relatively few studies partitioning that variation into its genetic and environmental components. Thus, it is relatively difficult or impossible to interpret which symbiotic component contributes to the phenotypic expression and any evolutionary significance of much of the fitness variation from such natural systems. The alternative approach of using symbiotic and nonsymbiotic clones (Arechavaleta et al., 1989; Hill et al., 1990; Hill et al., 1991a; Hill et al., 1991b; Agee and Hill, 1991) has greatly facilitated our approach and understanding of several aspects of the variation of mutualistic responses within a population. This approach should allow us to extrapolate to the effects of such variation on trophic interactions, one of which is measured as an antiquality factor. Examples of variation obtained from using clonal lines are indicated below.

In summary improved fitness includes increased growth rate and tiller density (Bradshaw, 1988; Hill et al., 1990; Hill et al., 1991a), changes in morphology (Diehl, 1950; Hill et al., 1990), resistance and toxicity to grazing animals (Byford, 1979; Wallner et al., 1983; Read and Camp, 1986), insect deterrence (Prestidge et al., 1982; Clay, 1988), nematode resistance (Kimmons et al., 1990; West et al., 1990a,b), disease resistance (White and Cole, 1985; Yoshihara et al., 1985), and drought tolerance (Read and Camp, 1986; Arechavaleta et al., 1989; Elmi and West, 1989). The chemical basis for each component of fitness is not completely understood, but data defining cause and effect relationships are eminent. There may be a chemical similarity between compounds which is responsible for each aspect of improved fitness although the compounds may be found in very distinct symbiota (Table 2). However, this information is far from complete as there are only a few experiments which document positive benefits from endophytes within other associations, particularly the balansioid symbiota.

All the positive benefits derived from the symbiota are expressed, or measured when the association is subjected to stresses. Characteristics which are associated with drought tolerance are not expressed unless the plant is exposed to prolonged drought conditions. For example, roots of symbiotic plants will grow faster and deeper into a soil profile under drought stress than its cloned nonsymbiotic ramet. However, there is no difference when both are grown at field capacity (Richardson et al., 1990). Further, total reserve carbohydrate content and structures are similar among plants, regardless of endophyte content, when soil water content is at field capacity (-0.1 bars), but sugar monomers increase and polymers decrease in symbiotic tall fescue once drought stress conditions are imposed (Richardson et al., 1992). Moreover, there is variation among symbiota, as for example, plant morphological changes are not constant among tall fescue genotypes (Hill et al., 1990). This variation is expressed specifically by tillering capacity, specific leaf weights, and crown weight which may increase, decrease, or remain the same depending upon the genotype of the infected plant. All of these factors may explain the basis of variation under stress.

Insect and Nematode Defense

The accumulation of antiquality components (Table 2) in symbiotic grasses is a clear example of a defensive mutualism derived from endophytic fungi. Detailed data expressing this are based on specific and generalized characteristics from insect feeding experiments, which suggest that at least antibiosis may be the functional defense mechanism. Since most insects are grazers, data obtained on insects may be appropriate for mammalian grazers. Thus, it is this category of mutualism which is directly related to the livestock toxicity syndromes discussed below. In the use of endophytes for forage improvements, we are dealing with a double edge sword: 1) a mutualism which is essential for the competitiveness of pasture and rangeland grasses, and 2) an antiquality component. The complex interaction described above must be considered if there are any attempts at removing the mammalian toxicity from the insect toxicity. This removal also may result in the total removal of all toxicity and related stress-sparing mechanisms.

There are numerous effects of symbiotic grasses on insect herbivory (Gaynor and Hunt, 1983; Barker, 1988; West et al., 1989; Siegel et al., 1991), and they suggest some specificity (Hardy et al., 1986; Siegel et al., 1991). The list of insects reported as being deterred or poisoned by symbiotic grasses includes several species of aphids (Latch et al., 1985a; Siegel et al., 1991), sod webworms (Funk et al., 1983), leafhoppers (Pottinger et al., 1985), chinch bugs (Funk et al., 1985), crickets (Asay et al., 1975; Ahmad et al.,

Table 2. Toxins and suspect toxins associated with symbiotic grasses.

Acremonioid symbiota	Balansioid symbiota
Ergovaline	Elymoclavine
Ergonovine	Ergonovine
Ergosine	Ergonine
Chanoclavine I	Chanoclavine I
Peramine	Isochanoclavine I
Indole acetic acid	Agroclavine
Ergosterol	Dihydroelymoclavine
Ergosinine	6,7-Secoagroclavine
Cyclopentanoid sesquiterpenoids*	Penniclavine
Ergonovine	Erythro 1-(3-indoly)propane-1,2,3-triol
Caffeic acid*	Threo 1-(3-indoly)propane-1,2,3-triol
p-Coumaric acid	3-Indole acetic acid
p-Hydroxybenzoic acid	3-Indole ethanol
Loline	3-Indole acetamide
N-acetylloline	Methyl-3-indolecarboxylate
N-formylloline	Ergobalansine
N-acetylnorloline	Ergobalansinine
Paxilline	
Lolitrem A	
Lolitrem B	
Lolitrem C	
Lolitrem D	

*These compounds were isolated from *Epichloe typhina (Acremonium typhinum)* and are grouped with the acremonioid symbiota. Hormane, norharmane, and halostachine were isolated from tall fescue (Yates, 1983), but the infection status of the grasses was not reported; therefore, they have been excluded from the list.

1985), corn flea beetle (Kirfman et al., 1986), black beetle (Siegel et al., 1987), bluegrass billbug (Ahmad et al., 1986), flour beetles (Clay, 1988), and Argentine stem weevil (Prestidge et al., 1982; Pottinger et al., 1985). Symbiotic grasses also are toxic to the lepidopteran larvae of fall armyworms (Hardy et al., 1985) and species of Crambis (Clay et al., 1985). Acremonioid grasses are reported as being nematicidal (Pedersen et al., 1988; Kimmons et al., 1990; West et al., 1990a), but this effect may be nulled if the symbiotic grasses also are associated with mycorrhizal infections (Barker, 1988).

Peramine is the chemical considered responsible for deterring the feeding activity of several insects, specifically the Argentine stem weevil (Rykard et al., 1985) and some species of aphids (Siegel et al., 1991). Peramine is a very simple alkaloid which has been reported in symbiotic perennial ryegrass and tall fescue (Rowan and Gaynor, 1986; Siegel et al., 1991). It is not known if the fungus makes this compound or if its synthesis requires a combination of fungus and grass. In addition to peramine, the loline alkaloids (N-formyl and N-acetyl loline) also have been implicated in the toxicity and deterring activity of one species of aphid, but affected another aphid species only if the infected grass contained other toxins (the ergopeptide alkaloids, peramine, and (or) the lolitrems) (Siegel et al., 1991). Several environmental factors also may be responsible for the final effect (McLean, 1970; Lyons et al, 1990a), and the co-occurrence of simple indoles, and terpinoids (Table 2) also may influence the toxicity.

Abiotic Stress Tolerances

Drought tolerance Most of the research conducted on the effects of endophyte infection on abiotic stress tolerances has been done on tall fescue. Similar mechanisms might exist for other *Acremonium*-infected grass of the same tribe, i.e., the perennial ryegrass symbiotum. However, it might be inappropriate to use infected tall fescue as a model for the warm-season *Balansia*-infected grasses. Nevertheless, the occurrence of insect and mammalian herbivore deterrences and disease resistance mechanisms in both types of symbiotic grasses suggest that similar abiotic stress mechanisms also might exist in the balansioid symbiota. Again, it is important in this concept to define, separate, and recognize the important contributions made by the fungus from that of the grass. Only when this separation is made can the total genetic potential of forage quality or antiquality be assessed.

Experimental evidence for the occurrence of a drought stress mechanism in symbiotic grasses is an outgrowth of the initial observation of Read and Camp (1986) that two of three populations of symbiotic tall fescue were more drought resistant than tall fescue plants with a low level of infection. Turgor maintenance has been identified as a major mechanism through which tall fescue tolerates drought (West et al., 1989), but root growth (De Battista et al., 1990; Richardson et al., 1990), and stomatal responses (Belesky et al., 1987; Richardson et al., 1992) also are considered important. While control mechanisms for drought tolerance in the tall fescue symbiotum are unknown,

it has been determined that only specific tissue types, young meristematic and elongating leaf, from the tall fescue symbiotum were capable of developing low osmotic potential in response to water stress (West et al., 1989). This suggests that the basic mechanism centers around the ability of grasses to develop low osmotic potential (West et al., 1990b). Further, since osmotic adjustment exists primarily in young and immature leaf blades, it may be the mechanism of persistence and tiller survival in the tall fescue symbiotum but only under intermittent drought (West et al., 1989; West et al., 1990b). The universal occurrence of this mechanism throughout populations of the acremonioid symbiota might vary. White et al. (1989) studied the expression of osmotic adjustment in two clones of the tall fescue symbiotum and concluded that cell wall elasticity appeared to explain the differences in turgor maintenance among the two clones.

The nature of the osmoticum responsible for this effect is unknown, but polyols, sugars, and amino acids are considered by many as likely candidates (Morgan, 1984). In addition to being non-metabolizable under stress, the essential substance or substances also must be osmotically active and compatible (nontoxic) with the normal plant physiological processes. Polyols were considered likely candidates since they are normal neutral metabolites of fungi, and have been reported as being osmotica in other plants (Lewis, 1967). In a study designed to examine the involvement of polyols in this mechanism, it was determined that in one genotype of symbiotic tall fescue, mannitol and arabitol were present (Richardson et al., 1992). However, the concentration of these polyols was not high enough to affect the overall osmotic pool in this genotype. It was further shown that in symbiotic grasses grown under drought stress, arabitol, glucose, and fructose were the only compounds which accumulated in sufficient quantities to affect the overall osmotic pool (Richardson et al., 1992).

Nitrogen Efficiency *Acremonium*-infected grasses appear to have some advantage over nonsymbiotic grasses in the area of nitrogen (N) utilization. The efficient utilization of low soil N by the tall fescue symbiota was reported by Arechavaleta et al. (1989). The amounts of dry matter produced by low N (11 mg/pot) were the same as the amount of dry matter produced by nonsymbiotic tall fescue at high (220 mg/pot), medium (73 mg/pot), and low (11 mg/pot) levels of N (Arechavaleta et al., 1989). Of the many enzymes responsible for N utilization, glutamine synthetase is primarily responsible for N efficiency. When the activity of this enzyme within the tall fescue symbiotum was compared to the activity in the uninfected plants grown under low N, it was discovered that glutamine synthetase was higher in the symbiotum. The high level of this enzyme in symbiota grown under low soil levels was considered an efficient means of utilizing N (Lyons et al., 1990b). The ability to efficiently utilize low N apparently is not present in seedlings of this symbiotum, but develops with grass maturity (Clay, 1987).

High levels of N not only increase the amount of dry matter produced in the tall fescue symbiotum (Arechavaleta et al., 1989; De Battista et al., 1990; Hill et al., 1990), but also the amounts of the ergot alkaloid (Lyons et al., 1986; Arechavaleta et al., 1989; Hill et al., 1990). Thus, although there is an increase in growth from N, the corresponding increase in the amount of toxins in the foliage should reduce herbivory. The effects of soil N on the accumulation pattern of other herbivore toxins are unknown, but since they contain N, they may also be affected by high soil N, as is observed for the accumulation pattern of the ergot alkaloids produced by *Claviceps* sp of rye.

As in the case of high soil N, fungal toxins might also interact with low soil N to deter herbivory. This is based on the concept that the total N content of plants affects the degree of insect herbivory (Mattson, 1980). Therefore, herbivore feeding on grasses is increased when grown at low soil N as compared to high soil N. Lyons et al. (1990b) determined that the total free N concentration of leaves of tall fescue was decreased significantly by endophyte infection although the plants were fertilized with high rates of N (10 Mm total N/pot). Under high N, increased herbivory is prevented due to the combined action of increased amounts of toxins and low total N content of herbage. In a mixed population of grasses, herbivory of symbiota would be less than herbivory of uninfected grasses. Thus, under a wide range of soil N levels, toxins and N content would offset any tendency to overgraze infected grasses.

Effects of Endophytes on Forage Quality

Quality-Antiquality Evaluation of Endophyte-Infected Grasses

The concept of grazing implies a very large category of trophic interactions but from the standpoint of livestock herbivory, it describes a trophic interaction with plants that results in both low lethality and intimacy of a forage grass. Of course, this is complicated by the fact that we are dealing with a natural phenomenon within the context of a man made and managed system. Grasses comprise most of a ruminant's diet and the selection of a grass species is based on its nutritive effects on livestock production. Analyses of several symbiotic and nonsymbiotic pasture or potential pasture grasses of tall fescue cultivars for nutritive values for livestock, as well as actual grazing trials, indicate that there are decided differences in actual livestock performance (Table 3) (Schmidt et al., 1982; Hoveland et al., 1983; Aldrich et al., 1990; Chestnut et al., 1992; Porter et al., 1993). However, laboratory analyses of symbiotic and nonsymbiotic tall fescue indicate that the two are identical in terms of protein, in vitro dry matter disappearance (IVDMD), fiber and lignin (Table 3), as well as mineral contents (Chestnut et al., 1991b).

The greatest impact of symbiotic grasses is on the low productivity of livestock, particularly ruminant livestock (Table 3). However, most of the symbiotic grasses also are used as conservation/recreational species and are

Table 3. Forage characteristics and cattle performance on symbiotic and nonsymbiotic tall fescue.

Item	Symbiotic	Nonsymbiotic	Ref*
Grass:			
IVDMD, %	65.8	62.6	1
Crude protein, %	9.2	8.2	2
Neutral detergent fiber, %	74.5	72.0	2
Acid detergent fiber, %	40.7	40.0	2
Ash, %	5.6	6.72	2
Cattle:			
Grazing days	768.0[a**]	593.0[b]	3
Beef gain (ha^{-1})	384.0[a]	492.0[b]	3
Average daily gain (Kg)	0.50[a]	0.83[b]	3
Gains per tester steer (Kg)	84.0[a]	144.0[b]	3
Rectal temperature (C)	40.1[a]	39.3[b]	1
Average feed intake (Kg/d)	4.40	4.79	1
Respiration rate, breath/min	55.0[a]	53.0[a]	1
Skin vaporization, kcal/m²/h	91.3	113.7	4
Prolactin, ng/ml	1.5	1.5	4
Triiodothyronine, ng/ml	57.2	60.6	4
Cortisol, ng/ml	57.2	60.6	4
Plasma melatonin, mean day-night difference, ng/ml	78.36[a]	48.10[b]	5

*References: [1]Schmidt et al., 1982; [2]Chestnut et al., 1991a; [3]Hoveland et al., 1983, 4 years means; [4]Aldrich et al., 1990; [5]Porter et al., 1993.

**Means in a row with the same letter are not significantly different at the $P < 0.05$ level.

grazed by wildlife. The effect of these grasses on wildlife resources is expected to mimic those observed in livestock production and small animal experiments. Thus, preliminary studies indicate that populations of small insectivorous and herbivorous mammals such as shrews, voles, and cotton rats were four to five-fold higher in pastures of nonsymbiotic tall fescue (Pelton et al., 1991). The effects of symbiotic grasses on deer, rabbits and other wildlife herbivores are sporadic and undocumented. As the emphasis on planting increased acreage of symbiotic grasses for turf and conservation purposes increases, the potential for increased toxicity and effects on wildlife also will increase.

***Acremonium* Toxicity: Tall Fescue** Historically, tall fescue and perennial ryegrass symbiota were associated with poor animal performance problems and toxicities. The most common of these are fescue toxicosis and ryegrass staggers (Cunningham, 1943; Yates, 1962; Fletcher and Harvey, 1982). Plants with deterrents to ruminant herbivory should have a competitive edge over plants without such a mechanism, thus selection would favor symbiotic grasses with such mechanisms over uninfected plants or symbiotic grasses without this mechanism. The degree of toxicity to ruminant herbivory is expected to reflect the amount of toxin contained within grasses. The amount of toxin within a plant at a given location varies both qualitatively (Yates et al., 1985; Lyons et al., 1986; Arechavaleta et al., 1991), and quantitatively (Bacon et al.,1986; Rowan and Shaw, 1987; Belesky et al., 1987b; Hill et al., 1991b) but fluctuates seasonally (Fluckiger et al., 1976; Belesky et al., 1987b; Belesky et al., 1988). Both genotypes of the grass and fungus affect the final expression of ergot alkaloid content (Hill et al., 1990; Hill et al., 1991b; Kearney et al., 1991). The variation and fluctuation of endophyte-infected tall fescue (Thompson et al., 1989a) within a location is expected to affect animal performance similarly (Crawford et al., 1989; Thompson et al., 1989b; Chestnut et al., 1991b).

Cattle consuming *A. coenophialum*-infected tall fescue may show either severe or mild symptoms. Cattle showing severe tall fescue toxicosis resemble those showing classical ergotism and include gangrene of the extremities, and a slight nervousness or palsy in the flank region. These symptoms, commonly referred to as fescue foot, are usually observed under cool temperatures. During the early studies of toxicity on this grass, these were the only signs recognized (Yates, 1962). Fescue foot was observed to affect only a few animals within the herd, and toxicity from tall fescue was not considered a serious economic threat, especially since it was so sporadic.

When the endophyte and the recognition of mild or subclinical effects on cattle were revealed (Schmidt et al., 1982), it became apparent that symbiotic tall fescue affected all animals within a herd and imposed severe economic losses (Stuedemann and Hoveland, 1988). The syndrome resulting from the subclinical effects often is referred to as fescue summer toxicosis or the summer slump, since it is more evident in the warm periods of the year. Cattle with the mild or subclinical symptoms exhibited reduced weight gains

and feed intake, reduced reproductive efficiency, reduced milk production, low heat tolerance or hyperthermia, increased respiration rates, and reduced circulating prolactin and serum cholesterol (Yates, 198; Porter et al., 1990).

Cattle affected with fescue summer toxicosis are further characterized as having low plasma melatonin (Porter and Thompson, 1992), rough and long hair coats which last well into the spring-summer period (Yates, 1983), and decreased tolerance to light (Hemken et al., 198; Bond et al., 1984). Cattle grazing symbiotic tall fescue seek shade or stand in water, and graze in the cooler periods of the day or at night. Another aspect of tall fescue summer toxicosis in cattle is the idiosyncratic development of fat necrosis which has been associated with high blood cholesterol of animals grazing symbiotic tall fescue (Stuedemann et al., 1985). Tall fescue summer toxicosis can be alleviated when the feed of cattle is changed, but average daily gains are still depressed if cattle are moved from symbiotic tall fescue to feedlots for finishing during warm weather.

Data showing that the greatest economic loss to the cattle industry is from the fescue summer toxicosis was estimated by Stuedemann and Hoveland (1988) to be $793 million annually in the USA which is primarily due to reduced reproduction rates and poor weight gains. According to Crawford et al. (1989), average daily gain of cattle was reduced by 45.5 g for each 10% increase in endophyte infestation level. The toxicity effects can be reduced by diluting the overall pasture infestation level with another forage such as clover (species of *Trifolium*) (Chestnut et al., 1991ab). In another study (Chestnut et al., 1991b), the average daily gains were depressed when the percentage of endophyte infestation levels was adjusted to 22% within by the addition of clover. This depression continued up to the 35% infection level, but no further reductions in average daily gain and beef production were observed when the infection was increased to 8?%, the highest level used (Chestnut et al., 1991b).

Horses grazing on *A. coenophialum*-infected tall fescue have very specific symptoms, particularly reproductive and neuroendocrine effects. These symptoms are, as in cattle, related to toxicity induced by ergot alkaloids and can be prevented by the daily administration of perphenazine, a synthetic dopamine antagonist (Ireland et al., 1989). There has been no report of the gangrenous aspect, fescue foot, occurring in horses. The endophyte lowers circulating progesterone and prolactin levels in brood mares which also have extended gestation periods, retained, mineralized and thickened placentas, agalactia, and deliver foals that are dysmature, weak, or stillborn (Monroe et al., 1992; Putnam et al., 1991; McCann et al., 1992). These symptoms are, as in cattle, related to toxicity induced by ergot alkaloids and can be prevented by the daily administration of perphenazine, a synthetic dopamine antagonist (Porter and Thompson, 1992).

Sheep grazing on *Acremonium*-infected tall fescue have lower circulating prolactin, cholesterol, and as much as a 59% reduction in milk production (Henson et al., 1987; Bond et al., 1988). Ewes have lowered conception rates, but unlike sheep grazed on perennial ryegrass, the growth rate and feed intake are not reduced (Bond et al., 1984; Bond et al., 1988).

***Acremonium* Toxicity: Perennial Ryegrass** Toxicity from perennial ryegrass infected with *A. lolii* affects mainly sheep (Keogh, 1973; Byford, 1979), but cattle, horses, and deer also are affected (Byford, 1979). This disease is a neuromuscular disorder and is referred to as ryegrass staggers (Keogh, 1973). The disorder occurs sporadically and primarily in New Zealand and southern Australia. It is distinct from the grass staggers (tetany) disease which is induced by a magnesium deficiency. It has been shown experimentally that sheep develop symptoms of ryegrass staggers within 7 to 14 d of being placed on toxic pastures (Fletcher, 1982). As is true of the symbiotic tall fescue, the syndrome in sheep is manifested in a variety of symptoms, including severe clinical symptoms of head nodding, trembling of the neck and shoulder muscles, swaying while standing, staggering, and a stilted gait with collapse if over-prodded (Keogh, 1973). Similar tetanic spasms are observed in cattle grazing perennial ryegrass; however, they may either collapse or assume a sitting posture if excited. These severe symptoms are associated with the level of endophyte (Hannah et al., 1990), increased amounts of dead basal dry matter in ryegrass stands, slow plant growth rate, or overgrazing (Keogh, 1973). There is a 2-10% loss of animals to ryegrass staggers which can account for at least half of the total profits. Subclinical effects include reduced average daily weight gains, depressed prolactin blood levels in ewes (Stilham et al., 1982; Fletcher and Barrel, 1984), and reduced testosterone levels in rams (Henson et al., 1987).

Symptoms of *Balansia* toxicity The *Balansia* species are mainly associated with invasive and rangeland grass species, e.g., species of *Sporobolus, Andropogon, Agrostis, Clamogrostis, Chloris, Eragrostis,* and *Panicum.* Detailed studies of their effects on grass quality and cattle performance have not been conducted. Since the balansioid endophytes produce ergot alkaloids similar to those produced by the acremonioid endophytes (Porter et al., 1979a,b; Porter et al., 1981; Lyons et al., 1986; Rowan and Shaw, 1987), their effects and modes of action on grazing animals should be the same (Witters et al., 1975; Berde and Schild, 1978). Indeed, ergotism in cattle has been reported in animals consuming *Balansia*-infected grasses (Hance, 1876; Nobindro, 1934; Bailey, 1903). Experimental feeding of the culture medium of one species, *B. epichloe*, reduced total prolactin concentrations in lactating Holstein cows (Wallner et al., 1983). Also, there was a decrease in the milk-induced rise in serum prolactin, although there was no effect on milk production (Wallner et al., 1983). This work was not only the first to establish that serum prolactin concentration was affected in cattle consuming a symbiotic grass but it also established that the ergot alkaloids produced by the fungus were the active toxins.

Toxins Associated with Symbiotic Grasses

The variety of defensive compounds (Table 2) found within symbiotic individuals of a population of endophyte-infected forage grasses will vary because of the process of natural selection under herbivory. Long-term herbivory, and specific types of herbivory (insect or mammalian), will lead to sub-populations of toxic grasses, each chemically defined and based on its specific mixture of deterring and toxic compounds. Since most endophytes are maternally transmitted, seed-sown pastures will reflect this diversity. This may serve to confound any potential cattle toxicities initially, but as cattle grazing within a location continues, the seed of toxic and deterring individual plants will be consumed the least, resulting in these individuals self-seeding, producing more of the toxic types.

The time required for the establishment of a population high in symbiotic individuals toxic to cattle may involve years. In areas where there are no pressures from herbivory, there should be a mixture of ecotypes, including individuals totally devoid of insect and mammalian toxins. Therefore, in experiments of short duration, it is important when studies of specific deterring or toxic mechanisms of symbiota are being conducted to consider this, and to define and use appropriate pastures. The major groups of toxins chemically identified with antiquality factors of symbiotic forage grasses are the ergot alkaloids and tremorgenic neurotoxins and these may be monitored to determine the percent distribution within a location.

The degree of chemical expression by the fungal mutualist depends not only on the biochemical competence of the fungus (Bacon et al., 1975; Bacon, 1988), but also upon the genotype of each plant (Hill et al., 1991b). The environmental factors of soil N (Gaynor and Hunt, 1983; Arechavaleta et al., 1989; Lyons et al., 1990b) and moisture (Arechavaleta et al., 1991) also interact to affect the accumulation of ergot alkaloids. Further, there is a fungus-grass genotype interaction since ergovaline content of one genotype of symbiotic tall fescue increases with increased leaf area while this relationship is not observed in other genotypes (Hill et al., 1990). This raises the issue as to whether the controlling mechanism is associated with the plant, the endophyte, or an interaction between the two. By inserting endophytes into a common tall fescue genotype and by conducting genetic studies between high- and low-ergovaline producing symbiotic plant genotypes (Johnson et al., 1986; Agee and Hill, 1991), it has been documented that at least the plant can regulate the expression of ergovaline production by the endophyte. In this regard, regulation may well reflect the individual variation of toxic precursors and primary metabolites released in the apoplasm from the plant. The apoplasm is the source of nutrients which are utilized by the fungi for growth and synthesis of secondary metabolites, many of which are toxic.

Ergot alkaloids The ergot alkaloids (Table 2) have been shown to be produced directly by the fungus as in tall fescue endophyte (Yates et al., 1985; Lyons et al., 1986), and the balansioid endophytes (Bacon et al., 1986), or indirectly in the case of perennial ryegrass since they are isolated only from symbiotic grasses (Rowan et al., 1986).

The ergot alkaloids found in symbiotic grasses consist of both the ergopeptine and clavine types (Porter et al., 1979b; Porter et al., 1981; Yates et al., 1985; Lyons et al., 1986). These two groups differ from each other in the presence (ergopeptide bond) or absence (clavine) of a peptide bond and attached amino acids. There is considerable controversy over the biological activity of each ergot alkaloid, but it is generally agreed that the ergopeptine alkaloids are more active than the clavine alkaloids.

The predominant ergot alkaloids found in the *Balansia*-infected grasses are of the clavine types, while the ergopeptide ergot alkaloids are found as the dominant ergot alkaloid in the *Acremonium*-infected grasses (Table 2). Both types of ergot alkaloids are biosynthetically derived from the same precursors, usually the simple clavine alkaloids (Floss, 1976), and there are biotypes of endophytes which reflect this variation (Bacon, 1988). Ergot alkaloids vary in concentration from 0.01 to 3.0 ug/g of plant (dry weight) (Gallager et al., 1984; Yates et al., 1985; Lyons et al., 1986) whose concentrations depend on the season, and the in planta physiological status (Bacon, 1988; Belesky et al., 1988), and grass-fungus interaction (Hill et al., 1991b; Agee and Hill, 1991). Livestock toxicity and possible mechanisms of action resulting from consuming toxic forages and specific ergot alkaloids have been reported in earlier reviews (Yates, 1983; Hemken et al., 1984; Porter and Thompson, 1992) and will not be repeated here.

Tremorgenic toxins The tremorgenic neurotoxins, commonly called the lolitrems, have been isolated only from the perennial ryegrass symbiotum. This group of toxins is apparently absent in symbiotic tall fescue and has not been examined for in the *Balansia*-infected grasses. This class of compounds is considered responsible for ryegrass staggers of sheep (Gallagher et al., 1982; Gallagher et al., 1984), and consists of four biologically active compounds, all containing a complex indole isoprenoid ring system (Gallagher et al., 1984). The major lolitrem is lolitrem B which ranges from 3 to 25 ug/g dry weight in perennial ryegrass herbage, and a smaller concentration also occurs in ryegrass seed (Gallagher et al., 1987). The lolitrems, unlike the ergot alkaloids, have not been isolated from cultures of the fungus *A. lolii*, but its indole isoprenoid precursor, paxilline, has, indicating that it is synthesized by the fungus in culture (Christopher and Mantle, 1987; Weedon and Mantle, 1987). In addition to finding paxilline in fungus cultures, it has been detected in ryegrass seed (Weedon and Mantle, 1987). Thus, it is unknown if the fungus only produces paxilline which is converted to the lolitrems by the plant, or if under culture or other conditions, there is an incomplete synthesis by the fungus. The latter might be the case, as other fungi can make tremorgenic neurotoxins, structurally related to the lolitrems, in culture (Lanigan et al., 1979) and otherwise (di Manna et al., 1976), which suggests that the *A. lolii* might be capable of synthesizing the lolitrem molecule in vivo but not in vitro.

Miscellaneous toxins There are a variety of chemically diverse compounds (Table 2) in symbiotic grasses which, as indicated above, were possibly one of the driving forces in evolution that led to the successful establishment of the associations. With the exception of the class of ergot alkaloids, there is presently no identified consistent and specific chemical which would suggest that there are other evolutionary biochemical relationships within the two broad types of symbiota.

The loline alkaloids are pyrrolizidine bases that are found in the tall fescue symbiotum in concentrations as high as 0.8% of the dry weight of tall fescue plants (Bush et al., 1982). These alkaloids have not been isolated from cultures of the fungus, and the production of similar pyrrolizidine bases by higher plants in general (McLean, 1970) imply that they may be products of the plant responding to infection from the endophyte. This implication is strengthened by the report of Belesky et al. (1987b), which indicates that the concentration of loline alkaloids reflects the extent of infection within the population. A limited number of studies suggests that the loline alkaloids are only mildly toxic (Bush et al., 1979; Strahan et al., 1988; Eichenseer et al., 1991) especially when that toxicity is compared with that of the usual pyrrolizidine alkaloids (McLean, 1970). Two of the forms present in symbiotic tall fescue, N-acetyl and N-formyl lolines, are considered more toxic than the other forms reported in this grass. However, these compounds may act synergistically or potentiate the activity of other toxins in symbiotic tall fescue, as suggested from their effects on insect herbivory (Siegel et al., 1991).

Other compounds reported in symbiotic grasses which may impose toxicity problems to livestock include 3-indole acetic acid, indole ethanol and related simple indoles (Porter et al., 1978), peramine (Rowan et al., 1986), the tetraenone steroid (Porter et al., 1975), the ergosterols (Davis et al., 1986), phenolic acid derivatives (Koshino et al., 1988), and sesquiterpenes (Yshihara et al., 1985). The effects of some of these on insects, laboratory animals, and fungi suggest toxicity (Yshihara et al., 1985; Davis et al., 1986; Dowd et al., 1988), while the toxicities of others have not been established.

PART II. MYCOTOXINS IN GRASSES FROM NONENDOPHYTIC FUNGI

Nonendophytic (nonmutualistic) fungi produce a wide diversity of mycotoxins (Cole and Cox, 1981) on many different substrates. However, the major focus of research on these toxins has been conducted on food commodities, although some emphasis has been placed on mixed animal feed. Thus, a complete listing of the presence or absence of mycotoxicoses, and (or) toxins occurring naturally on forage grasses is unavailable. The following discussion is based on information from the literature which indicates that the occurrence of a specific fungus taxon is found on grasses, although in certain

Table 4. Mycotoxins produced by nonendophytic fungi on or associated with growing or dried forage grasses.

Species	Mycotoxins	Animals affected	Forage type
Aspergillus: *A. fumigatus*	Fumigaclavines, fumigallin, fumigatin, fumitoxins, gliotoxin, fumitremorgens, verruculogen, ririditoxin, tryptoquivalines, and spinulosin	Cattle	Hay, grass litter
A. terrus	Citrovirdin, citrinin, gliotoxin, patulin, terreic acid, terretonin, territrems, cytochalasin E, and terrdionol	Cattle	Hay, leaves, stems
Claviceps: *C. purpurea, C. paspali, C. cynodontis*	Ergot alkaloids, and paspalinine	Cattle, pig, sheep, horses	Seed, seedheads,
Diplodia *maydis*	Diplodiatoxin	Cattle	
Fusarium: *F. sporotrichioides, F. poae, F. equisetti*	Butenolide, trichothecenes, zearalenone, and moniliforme	Cattle	Decaying leaves, leaves
Myrothecium: *M. roridum, M. leucotrichum, M. verrucaria*	Verucarins, and roridins	Sheep	Decaying leaves, rye stubble, hay
Penicillium: *P. crustosum, P. canescens, P. janczewskii*	Penitrem A, and penitrem B	Cattle	Litter
Phomopsis paspali	Cytochalasin H	Cattle	Litter
Pithomyces *chartarum*	Sporidesmins	Sheep	Ryegrass litter
Stachybotrys *chartarum (=atra)*	Epoxytrichothecene (Satratoxins)	Horses, pig, cattle, sheep	Hay, straw

cases the presence of its toxin has been inferred from either livestock behavior or demonstration of toxin production in culture by a fungus isolated

om grasses (Table 4). However, there are several livestock mycotoxicoses associated with forage grasses many of which are of considerable economic importance. Included among these are ergotism, paspalum staggers, stachybotoxicosis, facial eczema and geeldikkop, myrotheciotoxicosis, fusariotoxicoses, and toxicoses induced by *Penicillium* and *Aspergillus* species.

Grasses serving as substrates for nonendophytic fungi include most of the major forage species throughout the world, and these grasses may become toxic either during their period of active growth (field fungi) or while in storage (storage fungi). Mycotoxin accumulation occurs during or after fungal growth; therefore, the factors which favor growth indirectly or directly are also those that favor mycotoxin synthesis. Mycotoxins are secondary metabolites whose synthesis appears to depend on qualitative and quantitative nutritional factors during growth, the genetics of an isolate, and an interaction with one or more environmental factors. Detailed discussions of these factors as well as other mycotoxicoses on forage plants other than grasses are contained in earlier reviews (Ciegler et al., 1971; Kadis et al., 1971; Kadis et al., 1972; Purchase, 1974; Wyllie and Morehouse, 1977; Smith and Henderson, 1991).

Grass Mycotoxicoses

Ergotism

The genus *Claviceps* Tulasne is almost exclusively found on grasses throughout most of the temperate and tropical areas of the world. Species of *Claviceps* are field fungi and are phylogenetically related to the endophytes of grasses but differ in the absence of the endophytic habit. This fungus is a localized ovarian replacement disease in which the individual ovaries of florets are usually destroyed by the fungus. The fungus then occupies this location within the florets where it intercepts nutrients normally translocated to the ovary. There is also an indication that the sclerotium also competes for nutrients with noninfected florets, reducing the final yield of seed directly by replacement and by nutrient intervention (Bacon and Luttrell, 1982). During this very brief parasitic phase, the initial infective hyphae enlarges to ten times its initial size, often outweighing (wet weight) normal seed of a noninfected grass three-fold. The resulting mass of mycelium produced is referred to as a sclerotium. The sclerotium is the major location of toxins referred to as ergot alkaloids. These ergot alkaloids are similar, if not identical to those produced and described for the *Acremonium* endophytes. However, since species of *Claviceps* are localized to the flowering stage toxicity from this group can occur only by ingesting sclerotia. Leaves and stems of *Claviceps*-infected grasses are not toxic.

The genus *Claviceps* includes 26 species according to Langdon (1954). At least four more species have been named since this compilation, increasing this number to 30 (Walker, 1957 ; Pantidou, 1959; Kulkarni et al., 1976; Frederickson and Mantle, 1991). Species of *Claviceps* show three morphological levels of host associations. The lowest level resembles the balansioid endophytes described above, while the most advanced type is the typical dark-colored elongated sclerotial parasite, and is typified by *C. purpurea* (Fr.) Tul. The intermediate is typified by *C. paspali* Stev. and Hall, and is characterized by straw-colored globose-shaped sclerotia. Most of these species are associated with specific grasses, some of which are major forage species and they include: several species of fescue, bermudagrass (*Cynodon dactylon* (L.) Pers.), several species of *Paspalum* L., and a varied assortment of valuable rangeland species of perennial and annual grasses. *Claviceps purpurea* is one the most cosmopolitan species, occurring on well over 250 hosts of primarily temperate annual and perennial grasses. Three species (*C. nigricans* Tul., *C. grohii* Groves, and *C. junci* Adams) are associated only with the closely related relatives of grasses, the Juncaceae and Cyperaceae. The species of some grasses have developed physiological races with a wide host range; other species have narrow host ranges and also are characterized by a variable sclerotial size and morphology.

The species of *Claviceps* show considerable variation relative to alkaloid content and there is no correlation between grass host species and the capacity to produce ergot alkaloids neither quantitatively nor qualitatively. Individual sclerotia may or may not contain ergot alkaloids which may reflect either a fungal genetic or host and environmental interaction. The ergot alkaloids include many of those produced by the endophytes (Table 2) as well as several others.

The basic animal disorder of ergotism is usually characterized as gangrene of the extremities resulting from an impairment of peripheral circulation by the ergot alkaloids. Other aspects of ergotism include reproductive disfunction, and a nervous syndrome characterized by muscle tremors and uncoordinated movements when excited.

Paspalum Staggers

The dallisgrasses (*Paspalum* spp.) are found primarily in the tropical sections of the world. These grasses are parasitized specifically by *C. paspali*. In addition to producing the ergot alkaloids, this species also produces the tremorgenic substances collectively referred to as the paspalitrems (A, B, and C), and paspalinine (Table 4). These substances are produced in the sclerotia; thus, livestock toxicity results from ingesting infected seed heads. Ingested seed heads produce a neurological disease called paspalum staggers in cattle and sheep with clinical signs including tremors, incoordination, and ataxia. This disease occurs in Australia, Italy, New Zealand, Portugal, South Africa, and the United States. The neurological disease is not caused by and is distinct from similar appearing conditions caused by ingesting ergot alkaloids (Mantle et al., 1977). Paspalum staggers is caused by one of several diterpene indole-type compounds referred to as paspalinine and paspalitrems, of which paspalitrem B is the most biologically active (Cole et al., 1977; Gallagher et al., 1980).

Stachybotryotoxicosis

Stachybotryotoxicosis is primarily a disease of horses, but sheep, poultry, cattle, pigs, and man also may become affected. Animals show clinical signs ranging from death to mouth necrosis, salivation, edema, and inflammation of the head. It is caused by the cellulose saprophyte *Stachybotrys chartarum (Ehrenb. and Link) Huges* (=*S. atra, or S. alternans*) which colonizes straw of various grasses, although it also occurs on hay, and silage. Thus, this species should be considered a storage fungus. Sporadic outbreaks of this disease has been reported in Hungary, France, South Africa, Romania, Russia, Finland, the United States, and Czechoslovakia and it occurs primarily in the fall of the year. The toxins were identified by Epply (1977) as macrocyclic trichothecenes, commonly referred to as satratoxins F, G, and H.

Facial Eczema and Geeldikkop

Both facial eczema and geeldikkop are types of a photodermatitis reaction aggravated by sunlight and are distinguished from other forms of photodermatitis diseases in that the liver is damaged by the mycotoxin which prevents the detoxification of one of the porphyrin pigments formed by decomposition of chlorophyll (Mortimer and Ronaldson, 1983). The two phosensitizations are caused by toxins from spores of the same fungi but on different grasses under field conditions. Facial eczema is caused by *Pithomyces chartarum* (Berk. and Curt.) Ellis that colonize accumulated debris from perennial ryegrass during the autumn with subsequent sporulation and production of toxins occurring under low temperatures from February to May (Smith and Crawley, 1964; Brooks, 1969). Geeldikkop is associated with *P. chartarum* growing on debris of species of *Panicum*, although this disease is classically associated with this fungus on *Tribulus terrestris* L., an annual herb. It is considered that the host plants contribute to the basic differences of the two diseases. The identity of the mycotoxin in both diseases has been established as sporidesmin, a diketopiperazine. Almost all the toxin is contained within the spores which is taken in by livestock consuming *Pithomyces*-infected pasture litter. The major distinction between the two diseases is the degree of liver pathology which in geeldikkop is characterized by crystalloid substances in bile ducts (Coetzer et al., 1985). Facial eczema occurs mainly in sheep, although cattle and deer also are affected. Geeldikkop has been reported only in sheep and occurs mainly in South Africa (Coetzer et al., 1985) and southwestern United States (Sperry et al., 1955; Taber et al., 1968) where it occurs on *Panicum* species. On this latter grass species the mycotoxicoses then is referred to as dikoor.

Myriotheciotoxicosis

Two field fungi of the genus *Myrothecium* Kunze ex Fries, *M. roridum* and *M. verrucaria,* are associated with grasses, primarily perennial ryegrass, where they produce the toxic trichothecenes roridins (roridin A, D, E, and H) and verrucarins (Verrucarin A, B, and J) (Vertinskii et al., 1967; di Menna et al., 1973). These toxins were considered antibiotics by those who first isolated and determined their chemical structures (Brian et al., 1948; Harri et al., 1962). Both the roridins and the verrucarins are acutely toxic to a wide range of animals when administered orally, and can cause severe dermatitis if applied topically. These toxic fungi occur world wide in soil and are commonly found on the leaves of pasture grasses (di Menna et al., 1973). There is considerable variation in the ability of isolates to produce these mycotoxins and there are considerable toxicological variations that each isolate produces in experimental animals. Myrotheciotoxicosis is very similar to poisoning of cattle by kikuyu grass (*Pennisetum clandestinm* L.) (Martinovich et al., 1972) which suggests that the etiologic agent of this toxin is also a species of *Myrothecium*. Information on the natural outbreaks of this toxicoses are limited to New Zealand (di Menna et al., 1973; Mortimer et al., 1971). However, the widespread occurrence of these fungi, the ease with which it can colonize dead grass residues, and the potency of their toxins suggest that this taxon can play an important role in deteriorating the quality of grasses.

Fusariotoxicoses

The genus *Fusarium* Link consists of several species that produce toxins on grasses. Of the five most important toxigenic species (Marasas et al., 1984), three are considered important producers of toxins in pastures. These species are *F. sporotrichioides* Sherb., *F. poae* (Peck) Wollenw., and *F. equisetti* (Corda) Gordon. All three are cosmopolitan saprophytes that occur in soils and in grass debris. They are distributed from temperate to tropical and subtropical areas of the world. Of the three, *F. sporotrichioides* appears to be restricted to temperate and cold areas (Marasas et al., 1984). The toxins from these species produce problems in cattle, pigs, and horses.

The compilation of the fusariotoxins (Table 4) by the three *Fusarium* species of grasses indicates considerable diversity in chemical structure, yet these toxins or their modifications all are produced by the three taxons. Thus, the reason for combining the three species into one livestock disease complex as fusariotoxicoses. However, this presents a problem of actually assigning the correct fungus to the toxin and livestock disease. Appropriate identification can be achieved by isolating and identifying the fungus, although this too might present even greater problems since the identification of these fungi is difficult. For a more definitive description of these fungi and their toxicology the work of Marasas et al. (1984) should be consulted. A fourth species of the five, *F. moniliforme*, might become important, especially in the tropical areas of the world, if the extent of a single report of finding one of its toxins, fumonisin B_1 in a grass sample is corroborated to include other pasture areas of the world (Mirocha et al., 1992). Fumonisin B_1 is carcinogenic and considered responsible for swine pulmonary edema, leukoencephalomalacia in horses (Bezuidenhout et al, 1988; Marasas et al., 1988; Colven and Harrison, 1992). This mycotoxin is usually produced by this species on corn and corn products. This fungus also produces several other toxins (Bacon and Williamson, 1992), as well as other unknown toxins (Marasas et al., 1984)

Penicillium Toxicoses

The genus *Penicillium* Link consists of a large assortment of species found growing on a wide variety of food, feed, and plant residues. Members of this species, like those of the *Fusarium*, also are difficult to identify and several species have been reduced to synonymy. Thus, it is very difficult to correctly associate a known animal toxicoses with a specific *Penicillium* species with certainty. This large genus produce a wide variety of toxins but on forage grasses the number is greatly reduced. Table 4 lists three species and their major toxins isolated from grasses. These metabolites are neurotoxins and cattle toxicities from ingesting grasses infected with these species or their toxin have been reported (Gallagher et al., 1980; Wilson et al., 1981; di Menna and Mantle, 1978). The toxicity signs of livestock ingesting these toxins on grasses resemble those of other tremorgenic and staggers syndromes mentioned above. Indeed, isolation of the toxin might be necessary before a correct etiologic agent can be identified. Other tremorgenic toxins produced by these species are paxilline, verruculogen, verucosidin, and the janthitrems (Ciegler and Pitt, 1970). Documentation that these toxins are related to field cases of livestock toxicity is sporadic or lacking. Since *P. crustosum* Thom is very cosmopolitan and most isolates of it produce penitrem A (Pitt, 1979a), it might be the major species of concern for livestock toxicity from *Penicillium*-infected grasses.

Aspergillus Toxicoses

The genus *Aspergillus* Micheli was erected to describe those fungi that produce spores in chains which originate from a spore-bearing structure called a head. Fungi in this genus are primarily saprophytic and have the ability to grow on many different substrates under a range of environmental conditions (Pitt, 1979b). Two species, *A. fumigatus* Fresenius and *A. terreus* Thom, have been associated with livestock performance problems on aspergilli-infected grasses. However, the significance and natural frequency of occurrence of problems in livestock from these two aspergilli on grasses have not been well documented. Nevertheless, among the substrates which favor the growth of these *Aspergillus* species is decaying plant material, e.g., grasses. Further, both fungi are soil-borne and frequent colonizers of silage and hay. The important toxic aspergilli are listed in Table 4, and these toxins are mainly tremorgenic toxins. The important toxins include the fumitremogens A, B, and C produced by *A. fumigatus* (Yamazaki et al., 1971; Cole et al., 1977), and the territrems A, B, and C produced by *A. terreus* (Styne and Vleggaar, 1985). Other toxins produced by *A. fumigatus* include the clavine alkaloids fumigaclavines A and C, and the ergot alkaloids agroclavine, erymoclavine, festuclavine, and chanoclavine (Yamazaki et al., 1971). Both fungi are cosmopolitan and are frequent colonizers of hay, silage, and grass litter, especially under damp conditions (Cole et al., 1977). Toxicoses in livestock caused by *A. fumigatus* on grasses include abortions, tremors, and neurotoxicosis (Wilson et al., 1981), and liver and kidney damage (Thornton et al., 1968). Deaths and a protein deficiency syndrome also were attributed to *A. fumigatus* (Cole et al., 1977).

In addition to these two genera of aspergilli, an osmiophilic toxic species *A. chevalieri* (Mangin) Thom & Church is also found on several types of hay (Forgacs and Carll, 1962). Isolates of this species have been shown to produce physcion and other toxic anthraquinones (Bachmann et al., 1979). However, this taxon is not considered a serious problem since its toxins are not effective when they are administered orally.

SUMMARY

Several forage and rangeland grass species either formed a completely compatible association with a group of closely related fungi which are referred to collectively as endophytes, or they served as hosts to pathogenic or saprophytic fungal species. In both types of fungi, mycotoxins accumulate in forage and rangeland grasses. Mycotoxins are extrinsic antiquality factors in grass species. Mycotoxin accumulation occurs after and during fungal growth which may take place either during the growth phase of grasses or when grasses have been processed into hay or undergoing natural decay. Since mycotoxins are secondary rather than primary metabolites, their synthesis depends on qualitative and quantitative nutritional factors during and after growth, the genetic potential of the isolate, and an interaction with one or more environmental factors. These factors affect the accumulation pattern of toxins from both endophytic and nonendophytic fungi on grasses.

Control of the nonendophyte should be directed towards the removal of the fungus or conditions responsible for mycotoxin accumulation. Control of endophytes is complex. Control measures designed to prevent the growth of either endophytic or nonendophytic fungi must consider the specific ecological niche of a fungus. Successful prevention of mycotoxin accumulation implies not only a safe grazing product but also that all the agronomic aspects of forage quality have not been affected. Since endophytes increase the agronomic performance of grasses, e.g., by

prevention of overgrazing and increased stress tolerance they must be controlled differently from saprophytic and pathogenic fungal species.

Symbiotic associations of fungal endophytes with several grasses are defensive mutualisms. The consequence of this type of symbiosis is the production of a variety of chemicals, randomly distributed among forage grasses which are defensive chemicals to grazing ungulates, lagomorphs, rodents, as well as insects and several other invertebrates. While not all antiquality components have been analyzed in symbiotic grasses, those that have appear to serve both as deterrents and toxins and are considered to be affected by several physical environmental factors. Current concepts suggest that symbiotic grasses are natural deterrences to destructive grazing of forage and rangeland grasses. Additionally, drought tolerance, N efficiency, increased rooting and herbage yield also are attributed to this symbiotic association. However, relative to livestock farming, symbiotic grasses are egregious, and pose serious economic threats to grazing livestock. These economically important concerns include reduced reproductive efficiency, reduced weight gains, and often death of grazing livestock. The removal of mutualistic fungi from grasses may relieve one specific class of antiquality factors, mycotoxins, but the agronomic properties of the grass may be considerably impaired. This enigma can be solved by a recognition of the evolutionary significance of the mutualistic association, then genetically defining and partitioning desirable traits into single components, along with a concerted grass breeding effort designed at incorporating desirable genetic components into nonsymbiotic individuals and, if necessary, molecular modification of the desired forage if only by, for example, using transformed endophytes (Murray et al., 1992; Tsai et al., 1992).

REFERENCES

Agee, C.S., and N.S. Hill. 1991. Variability in progeny from high-and low-ergovaline producing tall fescue parents. p. 185. *In* Agronomy abstracts. ASA, Madison, WI.

Ahmad, S., S. Govindarajan, C.R. Funk, and J.M. Johnson-Cicalese. 1985. Fatality of house crickets on perennial ryegrass infected with a fungal endophyte. Entomol. Exp. Appl. 39:183-190.

Ahmad, S., J.M. Johnson-Cicalese, W.K. Dickson, and C.R. Funk. 1986. Endophyte-enhanced resistance in perennial ryegrass to the bluegrass bill-bug *Sphenophorus parvulus*. Entomol. Exp. Appl. 41:3-10.

Ahmadjian, V.,and S. Paracer. 1986. Symbiosis, an introduction of biological associations. University Press of New England, Hanover, NH.

Aldrich, C.G., M.T. Rhodes. J.L. Miner, J.A. Paterson, and M.S. Kerley. 1990. Effects of consumption of tall fescue infested with endophytic-fungus and supplementation with dopamine antagonist on heat dissipation, body temperatures and blood parameters in ruminants housed at elevated temperatures. p. 205-208. *In* S.S. Quisenberry and R.E. Joost (ed.) International Symposium on *Acremonium*/Grass Interactions. 5-6 Nov. 1990. Louisiana Agricultural Experiment Station, Baton Rouge.

Arechavaleta, M., C.W. Bacon, C.S. Hoveland, and D.E. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. 81:83-90.

Arechavaleta, M., C.W. Bacon, R.D. Plattner, C.S. Hoveland, and D.E. Radcliffe. 1991. Accumulation of ergopeptide alkaloids in endophyte-infected tall fescue grown under deficits of soil water and nitrogen fertilizer. Appl. Environ. Microbiol. 58:857-861.

ay, K.H., T.R. Minnick, G.B. Garner, and B.W. Harmon. 1975. Use of crickets in a bioassay of forage quality in tall fescue. Crop Sci. 5:585-588.

Bachmann, M., J. Luthy, and C. Schlatter. 1979. Toxicity and mutagenicity of molds of the *Aspergillus glaucus* group. J. Agric. Food Chem. 27:1342-1347.

Bacon, C.W. 1988. Procedure for isolating the endophyte from tall fescue and screening isolates for ergot alkaloids. Appl. Environ. Microbiol. 54:2615-2618.

Bacon, C.W. 1990. Isolation, culture and maintenance of endophytic fungi of grasses. p. 259-282. *In* D.P. Labeda (ed.) The isolation and screening of microorganisms from nature. McGraw-Hill, New York.

Bacon, C.W., and J. De Battista. 1991. Endophytic fungi of grasses. p. 231-256. *In* D.K. Arora (ed.) Advances in applied mycology. Marcel Dekker, Inc., New York.

Bacon, C.W., and N.S. Hill. 1994. Symptomless grass endophytes: Products of coevolutionary symbioses and their role in the ecological adaptation of infected grasses. *In* S.C. Redlin (ed.) Systematics, ecology and evolution of endophytic fungi in grasses and woody plants. American Phytopathological Society, St. Paul, MN. (In press)

Bacon, C.W., and E.S. Luttrell. 1982. Competition between ergots of *Claviceps purpurea* and rye seed for photosynthates. Phytopathology 72: 1332-1336.

characteristics of cattle grazing several varieties of tall fescue during summer conditions. J. Anim. Sci. 59:584-593.

Bradshaw, A.D. 1988. Population differentiation in *Agrostis tenuis* Sibth. II. The incidence and significance of infection by *Epichloe typhina*. New Phytol. 58:310-315.

Brian, P.W., H.G. Hemming, and E.G. Jeffreys. 1948. Production of antibiotics by species of *Myrothecium* in New Zealand pastures and its relation to animal diseases. J. Gen. Microbiol. 79:81-87.

Brooks, P.J. 1969. *Pithomyces chartarum* in pastures and measures for prevention of facial eczema. J. Stored Prod. Res. 5:203-207.

Bula, R.J., V.L. Lechtenberg, and D.A. Holt. 1977. Potential of the world's forage for ruminant animal production. p. 7-28. *In* Potential of temperate zone cultivated forages. Winrock International Livestock Research and Training Center, Morrilton, AR.

Bush, L.P., J. Boling, and S.G. Yates. 1979. Animal disorders. p. 247-292. *In* R.C. Buckner and L.P. Bush (ed.) Tall fescue. ASA, Maidson, WI.

Bush, L.P., P.C. Cornelius, R.C. Buckner, D.R. Varney, R.A. Chapman, P.B. Burrus, C.W. Kennedy, T.A. Jones, and M.J. Saunders. 1982. Association of N-acetyl loline and N-formyl loline with Epichloe typhina in tall fescue. Crop Sci. 22:941-943.

Byford, M.J. 1979. Ryegrass staggers in sheep and cattle. N. Z. J. Agric. May 1979:65.

Cheplick, G.P., and K. Clay. 1988. Acquired chemical defenses in grasses: The role of fungal endophytes. Oikos 52:309-318.

Chestnut, A.B., P.D. Anderson, M.A. Cochran, H.A. Fribourg, and K.D. Gwinn. 1992. Effects of hydrated sodium calcium aluminosilicate on fescue toxicosis and mineral absorption. J. Anim. Sci. 70:2838-2846.

Chestnut, A.B., H.A. Fribourg, J.B. McLaren, D.G. Keltner, B.B. Reddick, R.J. Carlisle, and M.C. Smith. 1991a. Effects of *Acremonium coenophialum* infestation, bermudagrass, and nitrogen fertilizer or clover on steers grazing tall fescue pastures. J. Prod. Agric. 4:208-213.

Chestnut, A.B., H.A. Fribourg, J.B. McLaren, R.W. Thompson, R.J. Carlisle, K.D. Gwinn, M.C. Dixon, and M.C. Smith. 1991b. Effect of endophyte infestation level and endophyte-free tall fescue cultivar on steer productivity. Tenn. Farm Home Sci. 160:38-44

Christopher, W.M., and P.G. Mantle. 1987. Paxilline biosynthesis by *Acremonium loliae*, a step toward defining the origin of lolitrem neurotoxins. Phytopathology 26:969-971.

Ciegler, A., S. Kadis, and S.J. Ajl (ed.). 1971. Microbial toxins. Vol. IV. Fungal toxins. Academic Press, New York.

Ciegler, A., and J.I. Pitt. 1970. Survey of the genus *Penicillium* for tremorgenic toxin production. Mycopathol. Mycol. Appl. 42:119-124.

Clay, K. 1984. The effect of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) on the reproductive system and demography of the grass *Danthonia spicata*. New Phytol. 98:165-175.

Clay, K. 1986. Grass endophytes. p. 188-204. *In* N.J. Fokkema and J. Van Den Heuvel (ed.) Microbiology of the phyllosphere. Cambridge University Press, Cambridge.

Clay, K. 1987. Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. Oecologia 73:358-362.

Clay, K. 1988. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. Ecology 69:10-16.

Clay, K., T.N. Hardy, and A.M. Hammond, Jr. 1985. Fungal endophytes of grasses and their effects on an insect herbivore. Oecologia 66:1-6.

Cockerell, T.D.A. 1956. The fossil flora of Florissant, Colorado. Amer. Mus. Natur. Histor. Bull. 24:71-110.

Coetzer, J.A., T.S. Kellerman, G.C.A. van der Weshuizen, W.F.O. Marasas, J.A. Minne, G.F. Bath, and P.A. Basson. 1985. The possible role of *Pithomyces chartarum* in the aetiology and pathogenesis of bovine hepatogenous photosensitivity disease in South Africa. p. 463-470. *In* J. Lacey (ed.) Trichothecenes and other mycotoxins. John Wiley and Sons, Chichester, England.

Cole, R.J., and R.H. Cox. 1981. Handbook of toxic fungal metabolites. Academic Press, New York.

Cole, R.J., J.W. Dorner, J.A. Lansden, R.H. Cox, C. Pape, B. Cunfer, S.S. Nicholson, and D.M. Bedell. 1977. Paspalum staggers: Isolation and identification of tremorgenic metabolites from sclerotia of *Claviceps paspali* stevens et Hall. Tet. Lett. 21:235-238.

Cole, R.J., J.W. Kirksey, J.W. Dorner, D.M. Wilson, J.C. Johnson, A.N.

Johnson, D.M. Bedell, J.P. Springer, K.K. Chetal, J.C. Clardy, and R.H. Cox. 1977. Mycotoxins produced by *Aspergillus fumigatus* isolated from moldy silage. J. Agric. Food Chem. 25:826-830.

Colvin, B.M., and L.R. Harrison. 1992. Fusarium-induced pulmonary and hydrothorax in swine. Mycopathologia 117:79-82.

Crawford, R.J., J.R. Forwood, R.L. Belyea, and G.B. Garner. 1989. Relationship between level of endophyte infection and cattle gains on tall fescue. J. Prod. Agric. 2:147-151.

Crowder, L.V. 1977. Potential of the world's forages for ruminant animal production. p. 49-78. *In* Potential of tropical zones cultivated forages. Winrock International Livestock Research and Training Center, Morrilton, AR.

Cunningham, I.J. 1943. A note on the cause of tall fescue lameness in cattle. N. Z. J. Sci. Technol. 24:167b-178b.

Davis, N.D., E.M. Clark, K.A. Schrey, and U.L. Diener. 1986. Steriod metabolites of *Acremonium coenophialum*, an endophyte of tall fescue. J. Agric. Food Chem.34:105-108.

De Bary, A. 1887. Comparative morphology and biology of fungi, mycetozoa and bacteria. Claredon Press, Oxford, UK.

De Battista, J.P., J.H. Bouton, C.W. Bacon, and M.R. Siegel. 1990. Rhizome and herbage production of endophyte-removed tall fescue clones and populations. Agron. J. 82:651-654.

Diehl, W.W. 1950. *Balansia* and Balansiae in America. p. 1-82. *In* USDA Agric. Monograph 4. U. S. Govt. Print. Office, Washington, DC.

di Menna, M.E., and P.G. Mantle. 1978. The role of *Penicillium* in ryegrass staggers. Res. Vet. Sci. 24:347.

di Menna, M.E., P.G. Mantle, and P.H. Mortimer. 1976. Experimental production of staggers syndrome in ruminants by a tremorgenic *Penicillium* from the soil. N.Z. Vet. J. 24:45-51.

di Menna, M.E., P.H. Mortimer, B.L. Smith, and M. Tulloch. 1973. The incidence of the genus *Myrothecium* in New Zealand pastures and its relation to animal diseases. J. Gen. Microbiol. 79:81-87.

Dowd, P.F., R.J. Cole, and R.F. Vesonder. 1988. Toxicity of selected tremorgenic mycotoxins and related compounds to *Spodotera frugiperda* and *Heliothis zea*. J. Antibiotics 61:1868-1872.

Eichenseer, H., D.L. Dahlman, and L.P. Bush. 1991. Influence of endophyte infection, plant age and harvest interval on *Rhopalosiphum padi* survival and its relation to quantity of N-formyl and N-acetyl loline in tall fescue. Entomol. Exp. Appl. 60:29-38.

Elmi, A.A., and C.P. West. 1989. Endophyte effect on leaf osmotic adjustments in tall fescue. p. 111. *In* Agronomy abstracts. ASA, Madison, WI.

Eppley, R.M. 1977. Chemistry of stachybotryotoxicosis, p. 285-293. *In* J.V. Rodrick, C.W. Hesseltine, and M.A. Mehlmann (ed). Mycotoxins in human and animal health. Pathotox Publishers, Park Forest South, IL.

Fletcher, L.R. 1982. Observation of ryegrass staggers in weaned lambs grazing different ryegrass pastures. N. Z. J. Exp. Agric. 10:203-207.

Fletcher, L.R., and G.K. Barrel. 1984. Reduced liveweight gains and serum prolactin levels in hoggets grazing ryegrasses containing *Lolium* endophyte. N. Z. Vet. J. 32:139-140.

Fletcher, L.R., and I.C. Harvey. 1982. An association of a *Lolium* endophyte with ryegrass staggers. N. Z. Vet. J. 29:185-186.

Floss, H. 1976. Biosynthesis of ergot alkaloids and related compounds. Tetrahedron 32:873-912.

Fluckiger, E., W. Doepfner, M. Marks, and W. Niederer. 1976. Effects of ergot alkaloids on the hypothalmic-pituitary axis. Postgrad. Med. J. 52:57-61.

Forgacs, J.,and W.T. Carll. 1962. Mycotoxicoses. Adv. Vet. Sci. 7:273-283.

Frederickson, D.E., and P.G. Mantle. 1991. *Claviceps africana* sp. nov.; the distinctive ergot pathogen of sorgham in Africa. Mycol. Res. 95:1101-1107.

Funk, C.R., P.M. Halisky, M.C. Johnson, M.R. Siegel, and A.V. Stewart. 1983. An endophytic fungus and resistance to sod webworms. Bio/technol. 1:189-191.

Funk, C.R., P.M. Halisky, S. Ahmad, and R.H. Hurley. 1985. How endophytes modify turgrass performance and response to insect pests in turfgrass breeding and evaluation trials. p. 137-145. *In* F. Lemaire (ed.) Proc. 5th International Research Conference, Avignon. Versailles:

Ireland, F.A., W.E. Loch, R.V. Anthony, and K. Worthy. 1989. The use of bromocriptine and perphenazine in the study of fescue toxicosis in pregnant pony mares. p. 201-206. *In* Eq. Nutr. Physiol. Symp. Proc., Stillwater, OK.

Johnson, M.C., L.P. Bush, and M.R. Siegel. 1986. Infection of tall fescue with *Acremonium coenophialum* by means of callus culture. Plant Dis. 70:380-382.

Kadis, S., A. Ciegler, and S.J. Ajl (ed.). 1971. Microbial toxins. Vol. VII. Algal and fungal toxins. Academic Press, New York.

Kadis, S., A. Ciegler, and S.J. Ajl (ed.). 1972. Microbial toxins. Vol. VIII. Fungal toxins. Academic Press, New York.

Kearney, J.F., W.A. Parrot, and N.S. Hill. 1991. Infection of somatic embryos of tall fescue with *Acremonium coenophialum*. Crop Sci. 31:979-984.

Keeler, K.H. 1985. Cost:benefit models of mutualism. p. 100-127. *In* The biology of mutualism:Ecology and evolution. Croom Helm, London.

Kelley, S.E., and K. Clay. 1987. Interspecific competitive interactions and the maintenance of genotypic variation within two perennial grasses. Evolution 41:92-103.

Keogh, R.G. 1973. Induction and prevention of ryegrass staggers in grazing sheep. N. Z. J. Exp. Agric. 1:55-57.

Kimmons, C.A., K.D. Gwinn, and E.C. Bernard. 1990. Nematode reproduction on endophyte-infected and endophyte-free tall fescue. Plant Dis. 74:757-761.

Kirfman, G.W., R.L. Brandenburg, and G.B. Garner. 1986. Relationship between insect abundance and endophyte infestation level in tall fescue in Missouri. J. Kans. Entomol. Soc. 59:552-554.

Kohlmeyer, J., and K. Kohlmeyer. 1974. Distribution of *Epichloe typhina* (Ascomycetes) and its parasitic fly. Mycologia 66:77-86.

Koshino, H., S. Terada, T. Yoshihara, S. Sakamura, T. Shimanuki, T. Sato, and A. Tajimi. 1988. Three phenolic acid derivatives from stromata of *Epichloe typhina* on *Phleum pratense*. Phytochemistry 27:1333-1338.

Kulkarni, B.G.P., V.S. Seshadri, and R.K. Hegde. 1976. The perfect stage of *Spacelia sorghi* McRae. Mysore J. Agric. Sci. 10:286-289.

Langdon, R.F.N. 1954. The origin and differentiation of *Claviceps* species. Univ. Queensland Papers, Dept. Botany 3:61-68.

Lanigan, G.W., A.L. Payne, and P.A. Cockrum. 1979. Production of tremorgenic toxins by *Penicillium janthinellum* Biourge: A possible etiological factor in ryegrass staggers. Aust. J. Exp. Biol. Med. Sci. 57:31-37.

Latch, G.C.M., M.J. Christensen, and G.J. Samuels. 1984. Five endophytes of *Lolium* and *Festuca* in New Zealand. Mycotaxon 20:535-550.

Latch, G.C.M., M.J. Christensen, and D.L. Gaynor. 1985a. Aphid detection of endophyte infection in tall fescue. N. Z. J. Agric. Res. 28:129-132.

Latch, G.C.M., W.F. Hunt, and D.R. Musgrave. 1985b. Endophytic fungi affect growth of perennial ryegrass. N. Z. J. Agric. Res. 28:165-168.

Latch, G.C.M., M.J. Christensen, and R.E. Hickson. 1988. Endophytes of annual and hybrid ryegrasses. N. Z. J. Agric. Res. 31:57-63.

Law, R. 1985. Evolution in a mutualistic environment. p. 145-170. *In* D.H. Bougher (ed.) The biology of mutualism: Ecology and evolution. Croom Helm, London.

Leuchtmann, A., and K. Clay. 1988. *Atkinsonella hypoxylon* and *Balansia cyperi*, epiphytic members of the Balansiae. Mycologia 80:192-199.

Lewis, D.H. 1967. Sugar alcohols (polyols) in fungi and green plants. New Phytol. 66:143-184.

Lewis, D.H. 1985. Symbiosis and mutualism: Crisp concepts and soggy semantics. p. 29-39. *In* D.H. Boucher (ed.) The Biology of mutualism. Oxford University Press, New York.

Luttrell, E.S. 1979. Host-parasite relationships and development of the ergot sclerotium in *Claviceps purpurea*. Can. J. Bot. 58:942-958.

Luttrell, E.S., and C.W. Bacon. 1977. Classification of *Myriogenospora* in the Clavicipitaceae. Can. J. Bot. 55:2090-2097.

Lyons, P.C., J.J. Evans, and C.W. Bacon. 1990a. Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. Plant Physiol. 92:726-732.

Lyons, P.C., J.J. Evans, and C.W. Bacon. 1990b. Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. Plant Physiol. 92:726-73[illegible].

Lyons, P.C., R.D. Plattner, and C.W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue. Science 2[illegible]:487-489.

Marasas, W.F.O., T.S. Kellerman, W.C.A. Glederblom, J.A.W. Coetzer, P.G. Thiel, and J.J. Van der Lugt. 1988. Leukoencephalomalacia in two horses induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet Res. 55:197-203.

Marasas, W.F.O., P.E. Nelson, and T.A. Tousson. 1984. Toxigenic *Fusarium* species. The Pennsylvania State University Press, University Park.

Mantle, P.G., P.H. Mortimer, and E.P. White. 1977. Mycotoxic tremogens of *Claviceps paspali* and *Penicillium cyclopium*: A comparative study of effects on sheep and cattle in relation to natural staggers syndrome. Res. Vet. Sci. 24:49-56.

Martinovich, D., P.H. Mortimer, and M.E. di Menna. 1972. Similarities between so-call kikuyu poisoning of cattle and two experimental mycotoxicoses. N.Z. Vet. J. 20:57-58.

Mattson, W.S. 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11:119-161.

McCann, J.S., A.B. Caudle, F.N. Thompson, J.A. Stuedemann, G.L. Heusner, and D.L. Thompson, Jr. 1992. Influence of endophyte-infected tall fescue on serum prolactin and progesterone in gravid mares. J. Anim. Sci. 70:217-223.

McLean, E.K. 1970. The toxic actions of pyrrolizidine (*Senecio*) alkaloids. Pharm. Rev. 22:429-483.

Mirocha, C.J., C.G. Mackintosh, U.A. Mirza, W. Xie, Y. Xu, and J. Chen. 1992. Occurrence of fumonisin in forage grass in New Zealand. Appl. Environ. Microbiol. 58:3196-3198.

Mohanty, N.N. 1964. Studies on ubatta disease of rice. Indian Phytopathology 17:308-316.

Monroe, J.L., D.L. Cross, L.W. Hudson, D.M. Hendricks, S.W. Kennedy, and W.C. Bridges. 1988. Effects of selenium and endophyte-contaminated fescue on the performance and reproduction in mares. Equine Vet. Sci. 8:148-153.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 3[illegible]:299-319.

Morgan-Jones, G., and W. Gams. 1982. Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloe typhina*, new taxa in one of two new sections of *Acremonium*. Mycotaxon 15:311-318.

Morgan-Jones, G., J.F. White, Jr., and E.L. Piontelli. 1990. *Acremonium chilense*, an undescribed endophyte occurring in *Dactylis glomerata* in Chile. Mycotaxon 39:441-445.

Mortimer, P.H., J. Cambell, M.E. di Menna, and E.P. White. 1971. Experimental myrotheciotoxicosis and poisoning in ruminants by verrucarin A and roridin A. Res. Vet. Sci. 12:508-515.

Mortimer, P.H., and J.W. Ronaldson. 1983. Fungal-toxin-induced photosensitization. p. 361-370. *In* R.J. Keeler and A.T. Tu (ed.). Handbook of natural toxins. Vol. 1, Plant and fungal toxins. Marcel Dekker, New York.

Murray, F.R., G.C.M. Latch, and D.B. Scott. 1992. Surrogate transformation of perennial ryegrass, *Lolium perenne*, using genetically modified *Acremonium* endophyte. Mol. Gen. Genet. 233:1-9.

Nelson, L.R., and J.C. Read. 1990. Fungal endophyte infection in Italian Ryegrass. Plant Dis. 74:183.

Nobindro, U. 1934. Grass poisoning among cattle and goats in Assam. Indian Vet. J. 10:235-236.

Pantidou, M.E. 1959. *Claviceps* from Zizania. Can. J. Bot. 37:1233-1236.

Pedersen, J.F., R. Rodriguez-Kabana, and R.A. Shelby. 1988. Ryegrass cultivars and endophyte in tall fescue affect nematodes in grass and succeeding soybean. Agron. J. 80:811-814.

Pelton, M.R., H.A. Fribourg, J.W. Laudre, and T.D. Reynolds. 1991. Preliminary assessment of small wild mammal populations in tall fescue habitats. Tenn. Farm Home Sci. 160:68-71.

Pitt, J.I. 1979a. *Penicillium crustosum* and *P. simplicissimum*, the correct names for two common species producing tremorgenic mycotoxins. Mycologia 71:1166-1177.

Pitt, J.I. 1979b. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, New York.

Porter, J.K., C.W. Bacon, and J.D. Robbins. 1979a. Ergosine, ergosinine, and chanoclavine I from *Epichloe typhina*. J. Agric. Food Chem. 27:595-598.

Porter, J.K., C.W. Bacon, and J.D. Robbins. 1979b. Lysergic acid amide derivatives from *Balansia epichloe* and *Balansia claviceps* (Clavicipitaceae). J. Nat. Prod. 42:309-314.

Porter, J.K., C.W. Bacon, J.D. Robbins, and D. Betowski. 1981. Ergot alkaloid identification in Clavicipitaceae systemic fungi of pasture grasses. J. Agric. Food Chem. 29:653-657.

Porter, J.K., J.D. Robbins, C.W. Bacon, and D.S. Himmelsbach. 1978. Determination of epimeric 1-(3-indoyl)propane-1,2,3-triol isolated from *Balansia epichloe*. Lloydia 41:43-49.

Porter, J.K., J.A. Stuedemann, F.N. Thompson, and L.B. Lipham. 1990. Neuroendocrine measurements in steers grazed on endophyte-infected fescue. J. Anim. Sci. 68:3285-3292.

Porter, J.K., J.A. Stuedemann, F.N. Thompson, B.A. Buchanan, and H.A. Tucker. 1993. Melatonin and pineal neurochemicals in steers grazed on endophyte-infected tall fescue: Effects of metoclopramide. J. Anim. Sci. 71:1526-1531.

Porter, J.K., and F.N. Thompson, Jr. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594-1603.

Porter. J.K., C.W. Bacon, J.D. Robbins, and H.C. Higman. 1975. A field indicator in plants associated with ergot-type toxicities in cattle. J. Agric. Food Chem. 23:771-775.

Pottinger, R.P., G.M. Barker, and R.A. Prestidge. 1985. A review of the relationships between endophytic fungi of grasses (*Acremonium* spp.) and Argentine stem weevil (*Listronotus bonariensis* Kuschel). p. 322-331. *In* Proc. 4th Australasian Conference Grassland Invertebrate Ecology. Lincoln College, Canterbury.

Prestidge, R.A., R.P. Pottinger, and G.M. Barker. 1982. An association of *Lolium* endophyte with ryegrass resistance to Argentine stem weevil. p. 119-122. In Proc. N. Z. Weed Pest Control Conf.

Purchase, I.H.F. (ed.). 1974. Mycotoxins. Elsevier, Amsterdam.

Putnam, M.R., D.I. Bransby, J. Schumacher, T.R. Boosinger, L. Bush, R.A. Shelby, J.T. Vaughan, D. Ball, and J.P. Brendemuehl. 1991. Effects of the fungal endophyte *Acremonium coenophialum* in fescue on pregnant mares and foal viability. Am. J. Vet. Res. 52:2071-2074.

Read, J.C., and B.J. Camp. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. Agron. J. 78:848-850.

Richardson, M.D., G.W. Chapman, Jr., C.S. Hoveland, and C.W. Bacon. 1992. Sugar alcohols in endophyte-infected tall fescue under drought. Crop Sci. 32:1060-1061.

Richardson, M.D., N.S. Hill, and C.S. Hoveland. 1990. Rooting patterns of endophyte infected tall fescue grown under drought stress. p. 129. *In* Agronomy abstracts. ASA, Madison, WI.

Rowan, D.D., and D.L. Gaynor. 1986. Isolation of feeding deterrents against Argentine stem weevil from ryegrass infected with the endophyte. J. Chem. Ecol. 12:647-658.

Rowan, D.D., M.B. Hunt, and D.L. Gaynor. 1986. Peramine, a novel insect feeding deterrent from ryegrass infected with the endophyte *Acremonium loliae*. J. Chem. Soc., Chem.Commun. 1986:935-936.

Rowan, D.D., and G.J. Shaw. 1987. Detection of ergopeptine alkaloids in endophyte-infected perennial ryegrass by tandem mass spectrometry. N. Z. Vet. J. 35:197-198.

Rykard, D.M. 1983. Comparative morphology of the conidial states and host-parasite relationship in members of Balansiae (Clavicipitaceae). Ph.D. diss. University of Georgia, Athens.

Rykard, D.M., C.W. Bacon, and E.S. Luttrell. 1985. Host relations of *Myriogenospora atramentosa* and *Balansia epichloe* (Clavicipitaceae). Phytopathology 75:950-956.

Rykard, D.M., E.S. Luttrell, and C.W. Bacon. 1984. Conidiogenesis and conidiomata in the Clavicipitoideae. Mycologia 76:1095-1103.

Sampson, K. 1933. The systemic infection of grasses by *Epichloe typhina* (Pers.) Tul. Trans. Br. Mycol. Soc. 18:30-47.

Schardl, C.L., J.S. Liu, J.F. White, Jr., R.A. Finkel, and M.R. Siegel. 1991. Molecular phylogenetic relationships of nonpathogenic grass mycosymbionts and clavicipitaceous plant pathogens. Plant Systemat. Evol. 178:27-41.

Schardl, C.L., and M.R. Siegel. 1993. Genetics of *Epichloe typhina* and

Acremonium coenophialum. p. 169-185. *In* R. Joost and S. Quisenberry (ed.) *Acremonium*/grass interactions. Vol.44, Agric. Ecosystem Environ., Elsevier Science Publishers, Netherlands.

Schmidt, S.P., C.S. Hoveland, E.M. Clark, N.D. Davis, L.A. Smith, H.W. Grimes, and J.L. Hilliman. 1982. Association of an endophytic fungus with fescue toxicity in steers fed Kentucky 31 tall fescue seed or hay. J. Anim. Sci. 55:1259-1263.

Siegel, M.R., G.C.M. Latch, and M.C. Johnson. 1987. Fungal endophyte of grasses. Ann. Rev. Phytopath. 25:293-315.

Siegel, M.R., G.C.M. Latch, L.P. Bush, N.F. Fammin, D.D. Rowen, B.A. Tapper, C.W. Bacon, and M.C. Johnson. 1991. Alkaloids and insecticidal activity of grasses infected with fungal endophytes. J. Chem. Ecol. 16:3301-3315.

Smith, J.D., and W.E. Crawley. 1964. Disturbance of pasture herbage and spore dispersal of *Pithomyces chartarum* (Berk. and Curt.) M.B. Ellis. N.Z. J. Agric. Res. 7:281-287.

Smith, J.E.,and R.S. Henderson. 1991. Mycotoxins and animal foods. CRC Press, Boca Raton, FL.

Sperry, O.E., R.D. Turk, G.O. Hoffman, and F.B. Stroud. 1955. Photosensitization of cattle in Texas. Texas Agric. Ext. Bull. 812.

Styne, P.S., and R. Vleggaar. 1985. The tryptoguivalines. Prog. Chem. Org. Nat. Prod. 48:62-65.

Stilham, W.D., C.J. Brown, L.B. Daniels, E.L. Piper, and H.E. Fetherstone. 1982. Toxic fescue linked to reduced milk output in ewes. Arkansas Farm Res. 31:9.

Strahan, S.R., R.W. Hemken, J.A. Jackson, Jr., R.C. Buckner, L.P. Bush, and M.R. Siegel. 1988. Performance of lactating dairy cows fed tall fescue forage. J. Dairy Sci. 70:1228-1231.

Stuedemann, J.A., and C.S. Hoveland. 1988. Fescue endophyte: History and impact on animal agriculture. J. Prod. Agric. 1:39-44.

Stuedemann, J.A., T.S. Rumsey, J. Bond, S.R. Wilkinson, L.P. Bush, D.J. Williams, and A.B. Caudle. 1985. Association of blood cholesterol with occurrence of fat necrosis in cows and tall fescue summer toxicosis in steers. Am. J. Vet. Res. 46:1990-1995.

Taber, R.A., R. Pittit, W.A. Taber, and J.W. Dollahite. 1968. Isolation of *Pithomyces chartarum* in Texas. Mycologia 60:727-730.

Thomasson, J.R. 1986. Fossil grasses: 1820-1986 and beyond. p. 159-167. *In* T.R. Soderstrom, K.W. Hilu, C.S. Campbell and M.E. Barkworth (ed.) Grass Systematics and Evolution. Smithsonian Institution Press, Washington, DC.

Thompson, R.W., H.A. Fribourg, and B.B. Reddick. 1989a. Sample intensity and timing for detecting *Acremonium coenophialum* in tall fescue pastures. Agron. J. 81:966-971.

Thompson, R.W., H.A. Fribourg, and B.B. Reddick. 1989b. Sampling intensity and timing for detecting *Acremonium coenophialum* in tall fescue. Agron. J. 81:966-971.

Thornton, R.H., G. Shirley, and R.M. Salisbury. 1968. A nephrotoxin from *Aspergillus fumigatus* and its possible relationship with New Zealand mucosal disease-like syndrome in cattle. N.Z. J. Agric. Res. 11:1-10.

Tsai, H.-F., M.R. Siegel, and C.L. Schardl. 1992. Transformation of *Acremonium coenophialum*, a protective fungal symbiont of the grass *Festuca arundinacea*. Curr. Genet. 22:399-406.

Vertinskii, K.I., K.A. Dzhilavyan, and V.P. Koroleva. 1967. A disease of sheep caused by the fungus *Myrothecium verrucaria* (In Russian). Byull. Inst. Eksp. Vet. 2:86-90.

Wallner, B.M., N.H. Booth, J.D. Robbins, C.W. Bacon, J.K. Porter, T.E. Kiser, R.W. Wilson, and B. Johnson. 1983. Effect of an endophytic fungus isolated from toxic pasture grass on serum prolactin concentrations in the lactating cow. Am. J. Vet. Res. 44:1317-1322.

Walker, J. 1957. A new species of *Claviceps* from Zizania. Can. J. Bot. 37:1233-1236.

Weedon, C.M., and P.G. Mantle. 1987. Paxilline biosynthesis by *Acremonium loliae*; a step towards defining the origin of lolitrem neurotoxins. Phytochemistry 26:969-971.

West, C.P., E. Izekor, A. Elmi, R.T. Robbins, and K.E. Turner. 1989. Endophyte effects on drought tolerance, nematode infestation and persistence of tall fescue. p. 23-27. *In* C.P. West (ed.) Proc. Arkansas Fescue Toxicosis Conference, No. 140. Arkansas Agric. Exp. Sta., University of Arkansas, Fayetteville.

West, C.P., E. Izekor, D.M. Oosterhuis, and R.T. Robbins. 1990a. The effect of *Acremonium coenophialum* on the growth and nematode infestation of tall fescue. Plant Soil 112:3-6.

West, C.P., D.M. Oosterhuis, and S.D. Wullschleger. 1990b. Osmotic adjustment in tissues of tall fescue in response to water deficit. Environ. Experimen. Bot. 30:1-8.

White, J.F., Jr. 1987. The widespread distribution of endophytes in the Poaceae. Plant Dis. 71:340-342.

White, J.F., Jr. 1988. Endophyte-host associations in forage grasses. XI. A proposal concerning origin and evolution. Mycologia 80:442-446.

White, J.F., Jr., and T.L. Bultman. 1987. Endophyte-host associations in forage grasses. VIII. Heterothallism in *Epichloe typhina*. Am. J.. Bot. 74:1716-1722.

White, J.F., Jr., and G.T. Cole. 1985. Endophyte-host associations in forage grasses. I. Distribution of fungal endophytes in some species of Lolium and *Festuca*. Mycologia 77:323-327.

White, J.F., Jr., and G.T. Cole. 1986. Endophyte-host associations in forage grasses. V. Occurrence of fungal endophytes in certain species of *Bromus* and *Poa*. Mycologia 78:102-107.

White, J.F., Jr., P.M. Halisky, S. Sun, G. Morgan-Jones, and C.R. Funk, Jr. 1992. Endophyte-host associations in grasses. XVI. Patterns of endophyte distribution in species of the tribe agrostideae. Am. J. Bot. 79:472-477.

White, J.F., Jr., A.C. Morrow, and G. Morgan-Jones. 1990. Endophyte-host associations in forage grasses. XII. A fungal endophyte of *Trichacne insularis* belonging to *Pseudocercosporella*. Mycologia 82:218-226.

White, R.H. 1989. Water relations characteristics of tall fescue as influenced by *Acremonium coenophialum*. p. 167. *In* Agronomy abstracts, ASA. Madison, WI.

Williams, M.J., P.A. Backman, E.M. Clark, and J.F. White, Jr. 1984. Seed treatments for control of the tall fescue endophyte *Acremonium coenophialum*. Plant Dis. 68:49-52.

Wilson, B.J., C.S. Byerly, and L.T. Burka. 1981. Neurologic disease of fungal origin in three herds of cattle. J. Am. Vet. Med. Assoc. 179:480-485.

Witters, W.L., R.A. Wilms, and R.D. Hood. 1975. Prenatal effects of elymoclavine administration and temperature stress. J. Anim. Sci. 41:1700-1704.

Wyllie, T.D., and L.G. Morehouse (ed.). 1977. Mycotoxic fungi, mycotoxins, and mycotoxicoses: An encyclopedic handbook. Vol. 1, 2, and 3. Marcel Dekker, New York.

Yates, S.G. 1962. Toxicity of tall fescue forage: A review. Econ. Bot. 16:295-303.

Yates, S. G. 1983. Tall fescue toxins. p. 249-273. *In* M. Recheigel (ed.) Handbook of naturally occurring food toxicants. CRC Press, Boca Raton, FL.

Yates, S.G., R.D. Plattner, and G.B. Garner. 1985. Detection of ergopeptine alkaloids in endophyte infected, toxic Ky-31 tall fescue by mass spectrometry/mass spectrometry. J. Agric. Food Chem. 33:719-722.

Yamazaki, M., S. Suzuki, and K. Miyaki. 1971. Tremorgenic toxins from *A. fumigatus*. Chem. Pharm. Bull. 19:1739-1742.

Yoshihara, T., S. Togiya, H. Koshino, S. Sakamura, T. Shimanuki, T. Sato, and A. Tajimi. 1985. Three fungitoxic sesquiterpenes from stromata of *Epichloe typhina*. Tetra. Let. 26:5551-5554.

Zahner, H., H. Anke, and T. Anke. 1983. Evolution and secondary pathways. p. 153-171. *In* J.W. Bennett and A. Ciegler (ed.) Secondary metabolism and differentiation in fungi. Marcel Dekker, New York.

APPENDIX II. TOXICITY IN *BRACHIARIA*

Summary statement

A number of researchers in Africa, SE Asia and tropical America have reported cases of toxicity (i.e. joundice, photosensitization, liver damage) in sheep, goats, cattle and horses fed with *Brachiaria* spp. The saprophytic fungus *Pithomyces chartarum* has been associated with the toxicity in *Brachiaria*. However, in many instances researchers have been unable to isolate spores of *P. chartarum* from *Brachiaria* pastures which had caused toxicity. In the cases where *P. chartarum* has been isolated from *Brachiaria*, the spore count has been low and would not account for the severe toxicity observed. Thus, the evidence available does not **conclusively** show that the toxicity problems in *Brachiaria* spp. is due to the fungus *P. chartarum*. Other fungi and plant metabolites (i.e. saponins, steroids) could be involved in cases of *Brachiaria* toxicity.

UNIDAD DE
DOCUMENTA

Plant populations failed to build up to a satisfactory density during any of the 5 years, despite a favourable initial establishment of approximately 80–100 plants m^{-2}. The plant count declined with time. It is most likely that the pattern of autumn and spring rainfall determined flowering and seed set and subsequent regeneration of the clover. The rainfall pattern over the 5 years was such that only minimal flowering and seeding occurred. Often the germinating rains were too late to permit flowering before the scorching summer heat. Sufficient seed must have been produced and/or there was sufficient hard seed carry-over in the drought season of 1982 to establish a nucleus colony in the following season.

Since clustered clover acts as an annual in south-east Queensland, a build up of seed is a prerequisite for regeneration and thickening of the stand. No evidence was obtained to suggest that poor persistence of the nodule bacteria was responsible for the slow build up of the clover stand. The dominant factor, therefore, appears to be the quantity and timing of the germinating rains which need to be adequate in either April/May or August/September to permit timely flowering and seeding in the cooler months of the growing season. This applied particularly in 1978 when seed was sown too late to gain advantage from good autumn and winter rain. Seed reserves may have been reduced also by "false strikes" in cool wet periods during summer because the young seedlings may not survive subsequent periods of high temperatures. Seedling mortality was seen in the district, particularly in January 1979 [illegible] inspected at that time.

Perhaps our initial seeding rate was below the threshold level for such a rigorous environment. Further investigations should examine whether speedier establishment may result from heavier seeding rates and/or resowing after an unsatisfactory first year seed setting.

Another limitation to seed build up may have been our failure to apply maintenance fertilizer and thereby promoting heavier seeding when climatic conditions were favourable.

ACKNOWLEDGEMENTS

We acknowledge the interest and assistance given by Messrs J. & G. Smith of "Alum Rock" on whose property the experiment was done.

REFERENCES

Brockwell, J., Schwinghamer, E. A. and Gault, R. R. (1977)—Ecological studies of root-nodule bacteria introduced into field environments. 5. A critical examination of the stability of antigenic and streptomycin-resistance markers for identification of *Rhizobium trifolii*. *Soil Biology Biochemistry* 9: 19–24.

Dudman, W. F. and Brockwell, J. (1968)—Ecological studies of root-nodule bacteria introduced into field environments—1. A survey of field performance of clover inoculants by gel immune diffusion serology. *Australian Journal of Agricultural Research* 19: 739–747.

Robinson, A. C. (1967)—The influence of host soil and rhizozphere populations of clover and lucerne root nodule bacteria in the field. *Journal of the Australian Institute of Agricultural Science* 33: 207–9.

Swann, I. F. (1972)—Stanthorpe Shire Handbook, Department of Primary Industries, Brisbane.

(Accepted for publication July 17, 1985)

TECHNICAL NOTE

PHOTOSENSITIZATION JAUNDICE SYNDROME IN WEST AFRICAN DWARF SHEEP AND GOATS GRAZED ON *BRACHIARIA DECUMBENS*

B. A. Opasina

International Livestock Centre for Africa (ILCA), c/o IITA, P.M.B. 5320, Ibadan, Nigeria

ABSTRACT

In a dry season grazing trial with Brachiaria decumbens *cv. Basilisk at Ibadan in southwest Nigeria, 14 from a group of 36 West African Dwarf sheep, and 1 from a group* *of 16 goats, died. The main clinical and post-mortem findings were signs of photosensitization and jaundice. Further investigation of the effect of the grass on ruminant production in Nigeria would be required.*

INTRODUCTION

Photosensitization has been observed among cattle and sheep grazed on *Brachiaria decumbens*, especially in South America (Andrade *et al.* 1978; Oliverra *et al.* 1979; CIAT 1980). It has not been reported amongst sheep and goats in Nigeria. In this paper, the author reports clinical and post-mortem findings in West African Dwarf sheep and goats grazed on pastures of *B. decumbens* in southwest Nigeria.

MATERIALS AND METHODS

Between late December 1981 and April 1982, 36 sheep (2 males and 34 females) and 16 female goats aged 6 to 15 months were grazed on pure pastures of *B. decumbens* cv. Basilisk, established in June 1981 on the ILCA farm at Ibadan in southwest Nigeria. The pasture was established from seed imported from Australia. The animals, which had been previously grazed on natural pastures at Fasola in Nigeria's derived savanna belt in the southwest, were given prophylactic measures against *Peste des Petits Ruminants* (PPR) and enterotoxaemia. They were also dewormed, treated against blood protozoa, and dipped against ectoparasites.

Three groups of sheep, containing approximately equal numbers, were grazed on pastures of *B. decumbens* located in different paddocks, whilst the fourth group was fed indoors (zero-grazed). Animals were allowed to roam in the open run attached to the sheds. The 16 goats were grazed separately from the sheep on pasture of *B. decumbens*. All animals were provided with mineral salt licks and fresh drinking water.

RESULTS

Ten days after the animals were introduced to the *B. decumbens* pastures, 58.3% of the sheep in the 4 paddocks showed some of the following symptoms: yellow colouration of the conjunctivae (38.9%) (sign of jaundice), conjunctivitis and bilateral corneal opacity (8.3%), exudative dermatitis with facial and body alopecia (27.8%), ataxia (13.9%), and paralysis of forelegs (8.3%) (Table 1).

Corneal opacity, exudative dermatitis, and paralysis were absent amongst the zero-grazed animals. Sheep in this paddock were relatively unexposed to sunshine as they were fed indoors. Exudative dermatitis, the overt sign of photosensitization, later

TABLE 1

Main symptoms observed among sheep in different paddocks of Brachiaria decumbens

Symptoms	Paddock: Pasture grazed I	II	III	Fed indoors IV	Total observation n	%
Yellow colouration of conjunctivae (jaundice)	5	4	3	2	14	38.9
Conjunctivitis and corneal opacity	2	1	—	—	3	8.3
Exudative dermatitis (including facial and body alopecia)	5	3	2	—	10	27.8
Ataxia	—	1	2	2	5	13.9
Paralysis (forelegs)	1	1	1	—	3	8.3
No. showing symptoms	10	5	4	2	21	
% showing symptoms	100	55.6	44.4	25.0		58.3
No. in each paddock	10	9	9	8	36	100

developed into longitudinal facial, spinal and leg alopecia in most cases. Alopecia of the periorbital area and roots of the ears were also observed in some of the animals.

Of the 16 goats exposed to the pasture, only 1 manifested toxicity symptoms, which appeared as periorbital alopecia, and yellow colouration of all the external mucous membranes (eyes, mouth and vulvovaginal area).

Amongst the sheep showing symptoms, the mean rectal temperature was 38.7 ± 0.7 °C and mean packed red cell volume (PCV) was 26.2 ± 4.7%. The only goat showing symptoms had a temperature of 38.8 °C and the PCV of 25%.

Ten days after the symptoms were first manifested, 14 out of 21 sheep showing toxicity symptoms died, twelve from the pasture-grazing group and two from the zero-grazing group (Table 2).

TABLE 2

Post-mortem findings among sheep in different paddocks of Brachiaria decumbens

Findings	Paddock: Pasture grazed I	Paddock: Pasture grazed II	Paddock: Pasture grazed III	Paddock: Fed indoors IV	n	Total affection: % of Affected animals	Total affection: % of Total animals
Dehydration	2	3	2	—	7	33.3	19.1
Yellow colouration of organs and tissues	5	4	3	2	14	66.7	38.9
Fatty liver	3	4	2	2	11	52.4	30.6
No. of sheep dead	3	5	4	2	14		
% death of total showing symptoms	30	100	100	100		66.6	
% death of total sheep	30	55.6	44.4	25.0			38.9

The gross pathological findings at post-mortem examination were generalised yellow colouration of organs and tissues (66.7%), fatty livers (52.4%), and dehydration (33.3%). The zero-grazed animals did not appear to be dehydrated. The only goat affected died showing marked generalised yellow colouration of the organs and tissues, fatty liver, and enlargement of the gall bladder.

DISCUSSION

The cause of the photosensitization jaundice syndrome is not known. Andrade *et al.* (1978) reported *Pithomyces chartarum*, a fungus cultured from pastures of *B. decumbens*, as the agent causing photosensitization among cattle and sheep exposed to the pasture. Fungal culture from *B. decumbens* pastures in this study did not yield *P. chartarum*, but yielded *Fusarium moniliforme*, another saprophytic fungus. Dosing sheep with *F. moniliforme* at a rate of 40 mg kg^{-1} body weight daily for 3 weeks failed to elicit the photosensitization jaundice syndrome observed under grazing conditions.

Neither exogenous nor endogenous sources of poisoning were observed in the pastures, although some of the symptoms resembled those of chronic copper poisoning observed by Edelsten (1980) among a government sheep flock at Marguba in northeastern Nigeria. The only suspected source of poisoning was through the salt lick supplementation, but the same salt lick blocks were supplied to other sheep and goats grazed on *Panicum maximum* on the same station with no untoward effects.

In this observation, neither bacterial nor protozoal infections were reported from blood and tissues submitted to the laboratory. Clinically, the animals did not show fever or anaemia and the mean packed cell volumes (PCV) were normal (Oduye 1976).

Since the syndrome observed was common to the animals in different paddocks, the causative agent is presumed to be associated with *B. decumbens* itself. Among the list of plants documented as the cause of photosensitization in domestic animals is a different species, *Brachiaria brizantha* (Clarke and Clarke 1975). In the trials reported here, with *Brachiaria decumbens*, photosensitization and jaundice were the main features. However, in Nigeria, these symptoms have not been reported in cattle. Although *Brachiaria decumbens* has been demonstrated to be the most promising grass in pure swards in northern Nigeria (Foster and Mundy 1961), further investigation would be required of its effects on cattle, sheep and goats.

ACKNOWLEDGEMENTS

I thank the Small Ruminant Programme of the International Livestock Centre for Africa, Ibadan, Nigeria, for permission to publish this paper.

REFERENCES

ANDRADE, S. O., DAS, S., BARROS, M. A., LEITE, G. G., DIAS, S. M. C., SAUERESSIG, M., NOBRE, D. and TEMPERINI, J. A. (1978)—Aspects of photosensitization in cattle on pasture of *Brachiaria decumbens* infected by *Pithomyces chartarum*. *Arquivos do Instituto Biologico* **45**: 117–136.

CIAT (1980)—Centro International de Agriculture Tropical (CIAT) Annual Report: Tropical Pastures Programme.

CLARKE, E. G. C. and CLARKE, M. L. (1975)—"Veterinary Toxicology" (Bailliere Tindall: London), p. 270

GEESTEN, R. M. (1980)—Chronic copper poisoning of sheep in Nigeria. *Tropical Animal Health and Production* **12**: 69–76.

FOSTER, W. H. and MUNDY, E. J. (1961)—Forage species in Northern Nigeria. *Tropical Agriculture* (Trinidad) **38**: 311–318.

ODUYE, O. O. (1976)—Haematological values of Nigerian goats and sheep. *Tropical Animal Health and Production* **8**: 131–136.

OLIVEIRA, G. P., NOAVES, A. P., COSTA, A. J. and ANDRADE, O. (1979)—Photosensitization in sheep caused by *Pithomyces chartarum* in pastures of *Brachiaria decumbens* in Sao Carlos region. *Cientifica* **7** (Special): 17–22.

(Accepted for publication 25 March, 1985)

PROCEEDINGS

FERTILIZERS FOR PASTURES—THEIR USE OR ABUSE

Canungra Field Meeting, May 10, 1985

The first field day of the Tropical Grasslands Society in 1985 was held on 10th May on the property of Mr. and Mrs. H. G. Benstead at Canungra. Talks were presented on the theme "Fertilizers for Pastures—their use or abuse" by various speakers, and then followed an inspection of irrigated river flats which had ryegrass/clover pastures oversown into kikuyu. These pastures had been treated with Roundup while one area was also deep ripped to alleviate soil compaction. Another area had been fertilized with nitrogen and sulphur, while another had received only nitrogen to demonstrate the sulphur problem on the farm.

INTRODUCTION—NEED FOR NUTRIENTS

B. WALKER

Department of Primary Industries, Brisbane

Twenty-five years or more of work has gone into fertilizer research for tropical pastures by CSIRO, DPI, Queensland University and commercial firms. By this time, we should have solved many of the problems, but unfortunately there is still a lot unknown and a lot of problems remain to be solved.

What has highlighted the concern about fertilizer use is that, of the 3.7 million hectares of improved pasture in Queensland (of which 1 million ha are legume/grass pastures), only 150 000 ha of this are being fertilized. This represents less than 10% of the total acreage.

37767

MARDI REPORT NO. 112

Tekuaird

Deleterious Effects of *Brachiaria decumbens* (Signal grass) on Ruminants

by
O. Abas Mazni and H. Sharif
Livestock Research Division, MARDI, Serdang, Selangor.

Diterbitkan oleh:
Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI)

Dicetak oleh:
Dr. Hj. Mohd. Yusof b. Hashim
Ketua Pengarah MARDI
Kementerian Pertanian Malaysia

September 1986

ISBN: 967-936-011-3

©Copyright Malaysian Agricultural Research and Development Institute, 1986.
First Published 1986.
All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission of MARDI.

Dicetak oleh Unit Percetakan MARDI, Serdang, Selangor
Kementerian Pertanian Malaysia.

DELETERIOUS EFFECTS OF *BRACHIARIA DECUMBENS* (SIGNAL GRASS) ON RUMINANTS

O. ABAS MAZNI and H. SHARIF

RINGKASAN

Sindrom penyakit kuning dan sensitif kepada cahaya adalah kesan daripada keracunan hati pada ternakan ruminan. Keracunan hati ini berlaku apabila ternakan hanya diberi makan rumput *Brachiaria decumbens*. Ciri-ciri penyakit ini ialah kerosakan hati dan seterusnya penurunan berat badan, penyakit kuning, sensitif kepada cahaya dan kematian. Kejadian penyakit ini pada lembu dan biri-biri dilaporkan berlaku di Colombia dan Brazil, Amerika Selatan, sementara di Malaysia penyakit ini dilaporkan hanya pada kambing dan biri-biri. Kesan keracunan pada kambing dan biri-biri adalah tinggi hingga menyebabkan kadar kematian mencapai 50% sementara kesan pada lembu di peringkat klinikal hanya setinggi 4.7 peratus. Selalunya ia berlaku pada lembu yang berumur antara sembilan hingga 24 bulan. Terdapat juga keracunan di peringkat subklinikal yang menyebabkan lembu kurus. Walaupun prinsip keracunan yang sebenar masih belum diketahui, gambaran mengenai penyakit ini adalah semakin jelas.

Kertas kerja ini melaporkan kejadian dan penyelidikan yang dijalankan mengenai sindrom penyakit kuning dan sensitif kepada cahaya yang disebabkan oleh keracunan hati berhubung dengan pemberian rumput *B. decumbens* sepenuhnya. Tanda-tanda penyakit, kajian patologi klinikal, postmortem dan histopatologi dibincangkan.

INTRODUCTION

The rearing of sheep and goats especially on small farmholdings in tropical and subtropical countries plays an important role in the production of meat. There is considerable potential in increasing the production of meat of these animals. Research on the nutrition and management of these animals is actively being carried out on large farms and among smallholders in Malaysia to increase the efficiency and productivity of these animals.

Availability of suitable pasture is a prerequisite towards achieving this goal. *Brachiaria decumbens* (Signal grass) which showed to be of agronomic potential as a pasture grass (WONG, 1980) has been extensively planted in all the livestock farms in Malaysia. It is one of the most productive and nutritive pastures for cattle in Colombia (TERGAS, PALADINES and KLEINHEISTERKAMP, 1983). This high yielding stoloniferous grass can adapt to a wide range of well-drained soils, grows well even with substandard management and during drought.

Cases of jaundice and photosensitization in cattle and sheep grazing on *B. decumbens* were reported in Colombia (ANON., 1978) and Brazil, South America (CARMAGO, WALTER, NELSON and REGINA, 1976; DOBEREINER, TOKARNIA, MONTEIRO, CRUZ, CARVALHO and PRIMO, 1976; NOBRE and ANDRADE, 1976; ANDRADE, LOPES, BARROS, LEITE, DIAS, SAUERESSIG, NOBRE and TEMPERINT, 1978; OLIVEIRA, NOVAES, COSTA and ANDRADE, 1979), Indonesia (TRIBUDHI, HELMY, VAN EYS, WILSON, PADERI and STOLZ, 1983) and Malaysia (ABAS MAZNI, MOHD. KHUSAHRY and SHEIKH OMAR, 1983; ABAS MAZNI, SHARIF, MOHD. KHUSAHRY and MOHD. ZAMRI, 1983). This paper reports on the jaundice and photosensitization syndrome associated with *B. decumbens* with reference to literature as well as the authors' observations and trials.

THE PLANT – *BRACHIARIA DECUMBENS*

The genus *Brachiaria* includes a number of species used by pastoralists in the tropics. In addition to several annual species widely distributed in tropical Africa, six perennial species – *B. brizantha, B. decumbens, B. distyoneura, B. humidicola, B. mutica* and *B. ruziziensis* have been used in tropical pastures with varying degrees of success. *Brachiaria decumbens (Plate I)* is widely spread both in open grasslands and partial shade on Great Lakes Plateau in Uganda and adjoining countries of east and central Africa (CATFORD, 1952; DOUGALL and BOGDAN, 1958; RATTRAY, 1960). It is one of the more widely used and of recent years, increasing interest has been shown by a number of tropical countries especially Brazil, India (JUDD, 1975), Colombia (BLASCO and BOHOROUEZ, 1967), Jamaica (RICHARDS, 1970), Venezuela (MILLER, 1974), Peru (SANTHIRASEGARAM, DIEZ, PETERSEN and TRIGUEROS, 1974), Northern Australia (MACKAY; 1974; STURTZ, HARRISON and FALVEY, 1975; WINTER, 1976) and Malaysia (WONG, 1980). Its high productivity, tolerance to low soil fertility conditions, drought resistance and relative freedom from pests and diseases account for much of the current interest (LOCH, 1977).

*Plate 1. The plant — **Brachiaria decumbens** (Signal grass)*

INCIDENCES OF JAUNDICE AND PHOTOSENSITIZATION IN OTHER COUNTRIES

Brachiaria decumbens and *B. brizantha* were introduced into Australia in the 1980s from central East Africa. In the 1940s botanist recognized *B. brizantha* as a separate species but some taxonomists considered it as only one end of the continuum with *B. decumbens* as the other end was proven very promising in CSIRO, Lawes, Queensland (WOOLCOCK, *pers comm.*, 1983). According to BRITON and PALTRIDGE (1940), sheep showed photosensitization when they grazed on pure stand of *B. brizantha*. This grass was then listed as a cause of liver disorder in sheep which was accompanied by hepatic dysfunction, photosensitization and icterus (CLARE, 1952). The work on this grass ceased abruptly after the incidence and sowing of this grass for pature was discouraged (WOOLCOCK, *pers comm.*, 1983).

Brachiaria decumbens was widely found in north coast wet tropic of Australia particularly around the south Johnstone, Tully and Mareeba areas (WOOLCOCK, *pers comm.*, 1983). Most of the pasture were planted commercially for the production of seed for exportation. In Queensland, there were cases of jaundice and photosensitization occurring in cattle grazing *B. decumbens*. The grass could not be conclusively said to cause the syndrome as the stock (cattle) had also access to lantana which gave similar syndrome (HOPKINSON, *pers comm.*, 1983). In Jamaica, continuous grazing of pure swards of *B. decumbens* had caused a form of scouring, resulting in poor animal performance (RICHARDS, 1970).

A similar syndrome occurring in sheep and cattle grazing on *B. decumbens* had been described in tropical areas of Latin America. The first report of photosensitivity in South America was made in Colombia in 1975. In 1977, in the incidences at Carimagua, Colombia two heifers (less than 24 months) out of a group of 20 grazing on this pasture showed marked photosensitization lesions on the skin. The lesions started to recede after the animals were removed from the pasture. At the same place, 16 out of 123 sheep died with signs of swollen faces, oedema, weakness and anaemia within three weeks after they were introduced into *B. decumbens* pasture. No more death occurred with these clinical signs once the animals were removed from the pasture. Many farms in Carimagua and other farms in the Llanos Orientales of Colombia with *B. decumbens* reported cases of photosensitivity (ANON., 1978; 1979). In Brazil, similar incidences occurred in cattle and sheep grazing on this grass and the fungus, *Pithomyces chartarum* had been mentioned to be related to the syndrome. (CARMAGO *et al.*, 1976; DOBEREINER *et al.*, 1976; NOBRE and ANDRADE, 1976; ANDRADE *et al.*, 1978; OLIVEIRA *et al.*, 1979). In both countries, it appeared that the intoxication produced by this forage affected only a small proportion of the cattle (1% to 5%). It occurred primarily on cattle below 14 months of age and the affected animals gradually recover if they were moved to other pastures soon after showing signs of photosensitivity (ANON., 1980). Due to photosensitization in young cattle which occurred between nine and 24 months of age, the use of this pasture declined. Cases in cattle in 1981 had two commonly seen clinical manifestations. The animals developed oedema in the lower neck, dewlap and ears. This oedematous form is apparently more acute and usually terminates in death of affected animals. In the skin necrosis form, the animals developed severe necrosis in several areas of the skin, but most frequently in the perineal region, abdomen and lower part of the neck. In all these cases, there was obvious liver involvement detected by gross lesions and high levels of gamma–glutamyl transferase (GGT) and serum glutamate oxaloacetate transaminase (SGOT) enzyme. Animals developed generalized icterus and enlarged liver which is the clinical and pathological changes of hepatic damage manifestations. Clinical cases were common in only 4.7% of affected cattle (GARCIA, AYCARDI, ZULUAGA, RIVERS and HENAO, 1982).

When hepatic damage was evident in animals grazing *B. decumbens*, a significant proportion lost weight before developing external photosensitization (ANON., 1981). A close association was shown to exist between underaverage weight gain animals and the presence of subclinical photosensitization. Thus the economic importance of this syndrome might be larger than previously expected. On the other hand, there were some evidence that photosensitization might also be related to very low levels of zinc in *B. decumbens* (ANON., 1982).

INCIDENCES OF JAUNDICE AND PHOTOSENSITIZATION IN MALAYSIA

MARDI Farm, Serdang

The first incidence of jaundice and photosensitization was observed in 1975 occurring in six Kedah-Kelantan cattle grazing on pure stand of *B. decumbens* in the Malaysian Agricultural Research and Development Institute (MARDI) farm (CHEN, *pers comm.*, 1982). In the incident, it was observed that the photosensitization appeared to be of hepatogenous origin as evident by toxic damage of the liver cell. Field investigation revealed that the pasture consisted of only *B. decumbens*. It was then postulated that the hepatotoxic material originated likely from the plant. Since then, there were other suspected cases occurring in Sahiwal-Friesian, Hereford and Local Indian Dairy (LID) cattle. Similar incidence involving six Kedah-Kelantan cattle was also observed in MARDI Station, Kluang. The clinical signs observed were icterus and extensive skin sloughing on the dorsal part of the body indicating photosensitization *(Plate 2)*. (DAHLAN, *pers comm.*, 1982).

Sheep and goats grazing on *B. decumbens* were also observed showing similar signs. The first two incidences occurred in 1979 in the goat and sheep farm, MARDI Station, Serdang (SUPARJO and ABDUL WAHID, 1980). Another incidence occurred in 1980 in which sheep showed the above clinical signs ten days after continuous grazing. There were no new cases once the animals were taken out of the pasture.

Pusat Pembiakan Kambing dan Biri-biri, Gajah Mati, Kedah

Incidences of jaundice and photosensitization were observed in goats and sheep as early as 1979 on this farm. There were high mortalities throughout the years since 1976, averaging 40% per year. Out of 70 postmortems submitted between 1979 and 1982 to the diagnostic

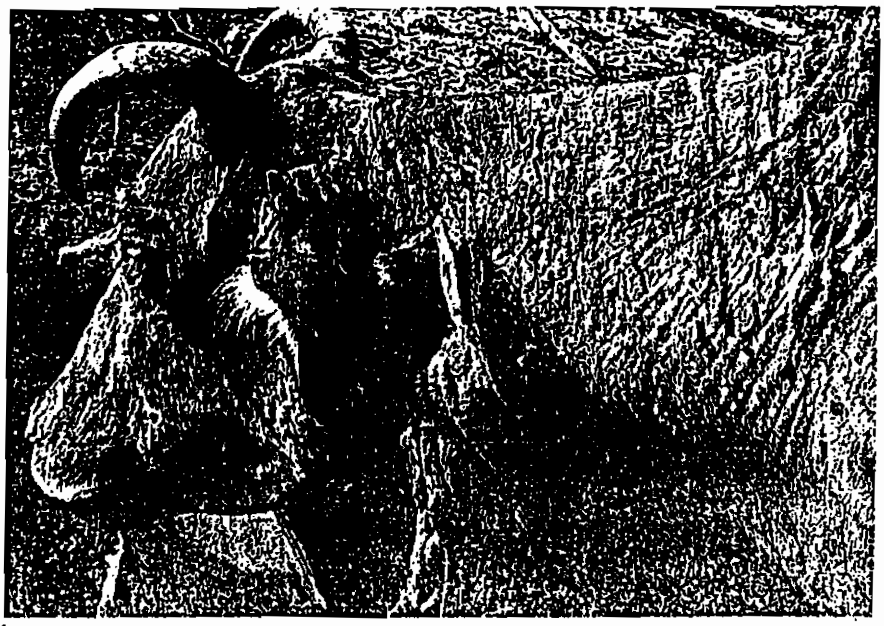


Plate 2. Photosensitization (skin sloughing) in Kedah-Kelantan cattle grazing on Brachiaria decumbens.

laboratory, 58.5% were seen with jaundice, haemolytic crisis and photosensitization. In the year 1979 when incidences of jaundice were high, the mortality rate was as high as 56.6% (ABAS MAZNI and SHARIF, 1983). Postmortem was not done in most of the cases since they showed similar signs (HANIFAH, *pers comm.*, 1981). The cases persisted until 1981. After the 1981 incidence, the animals were not fed this grass totally but instead rotational grazing on different pastures were carried out. Since then few cases occurred sporadically but the animals recovered due to early detection and treatment. In January 1983 similar cases occurred in a group of 40 indigenous sheep of Malaysia (ISM) belonging to Department of Veterinary Services, Kedah after 29 days of grazing *B. decumbens* (CHE AZIZ, *pers comm.*, [illegible]).

The incidence in Gajah Mati occurred mainly in newly bought goats and sheep. Newly bought Dorset Horn were also affected. Sheep gave earlier and more severe signs usually two weeks after grazing. Postmortem revealed acute toxic hepatitis with hepatolobular necrosis. This was assumed to be the predisposing factor to the jaundice and photosensitization syndrome. In some of the animals, there were signs of haemolytic crisis and glomerulonephrosis which occurred concurrently. The etiological agent of this toxicity was not fully determined.

Pusat Pembiakan Kambing, Kuala Pah, Jelebu, Negeri Sembilan

In March 1981, ten out of 39 goats died in this newly established goat farm and they were reported showing signs of jaundice (ABDUL MANAF, *pers comm.*, 1983). *Brachiaria decumbens* was then the major pasture available. Such cases were less frequently seen once the animals were let to graze on other pasture grasses. In late 1983, few incidences occurred when goats were fed *B. decumbens* due to lack of grass. No new cases were observed once the animals were removed from this pasture.

Smallholder Sheep Integration Research, Bachok, Kelantan

A smallholder sheep integration research under coconut was carried out by MARDI in Bachok, Kelantan in May 1982. Thirty-two ISM were given to two smallholders where 16 animals were let to graze on natural grass while the rest grazed on *B. decumbens*. Within a month, eight sheep which were grazing on *B. decumbens* showed signs of jaundice and photosensitization as indicated by shedding of wool and skin sloughing around the body, eye, muzzle and ears *(Plate 3)* (LIM, *pers comm.*, 1982). These animals died while the other seven from this group showed high levels of GGT and SGOT enzyme levels reflecting liver damage. The condition of these animals improved once they were removed from the pasture. None of the animals grazing on natural grass showed signs of liver damage.

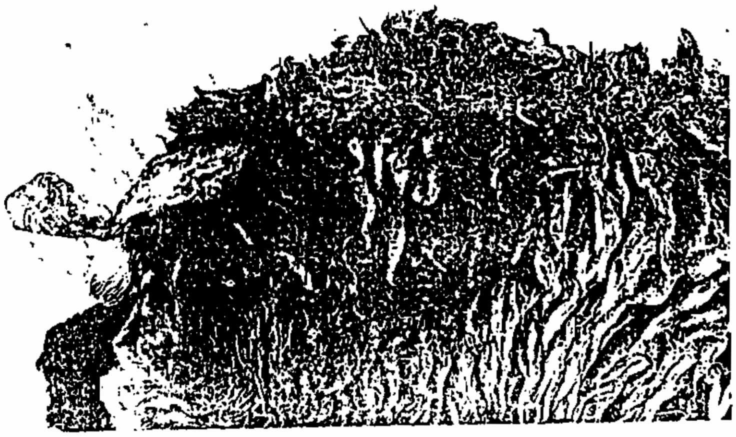
*Plate 3. Clinical signs of **Brachiaria decumbens** toxicity in indigenous sheep of Malaysia*

3a. Ulcerative dermatitis around the muzzle, eyelids, lips and ear.

3b. Shedding of wool with skin sloughing.

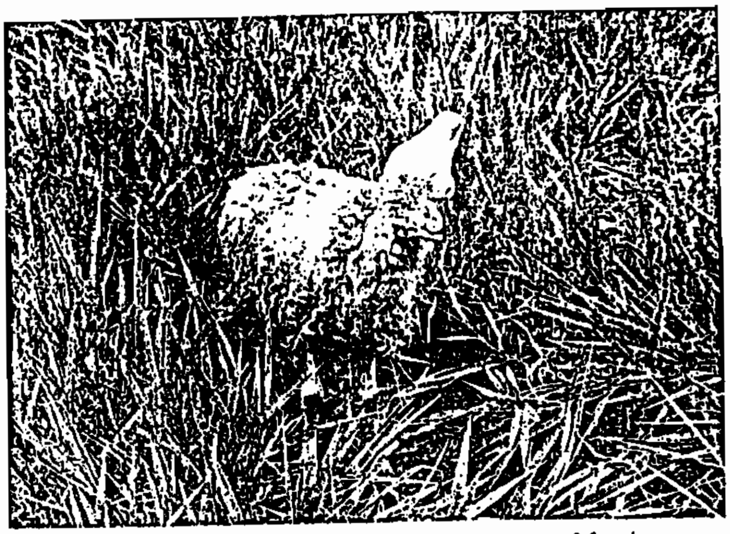
3c. Oedema of the ear causing it to droop.

3d. Ulceration of the skin around the eye.



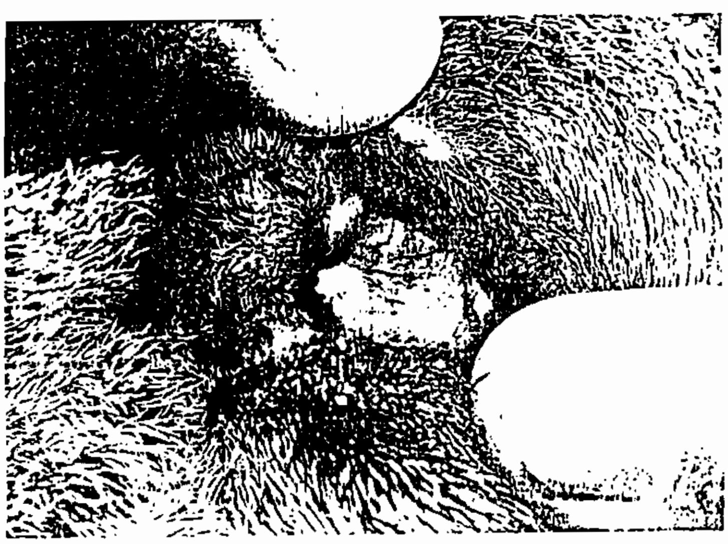
3e. Skin sloughing on the white patch of the tail.

3f. Wool shedding



3g. Star grazing, head shaking and stamping of forelegs.

3h. Bilateral eye opacity.



3i. Jaundiced third eyelids.

3j. Jaundiced and oedematous vulva. With prolonged exposure to sunlight the vulva becomes ulcerated.

Pusat Ternakan Kambing, Batu Arang, Selangor

A sudden outbreak of this syndrome occurred in August 1982 in different breeds of goats on this farm. It occurred only after animals were let to graze continuously on the newly established *B. decumbens* which covered 80% of the total pasture land. Out of the 165 goat carcasses submitted (June 1982 to June 1983) to Makmal Diagnosa Veterinar, Petaling Jaya, 60% of them were jaundiced carcasses (SHAHIRUDIN, TAN and RAJENDRAM, 1983). Copper toxicity was suggested to be the possible cause of this syndrome. However, in this case only 11 out of 79 or 13.9% of the liver samples submitted showed high level of copper above 500 parts per million. This indicated, that only a small fraction of the jaundice cases could be associated with copper toxicity. The copper toxicity in this case was possibly secondary to the liver damage, caused by *B. decumbens*.

There was an indication that Saanen was very susceptible to this intoxication, since 80% of these animals that were imported from Australia died within five months, a majority of which showed jaundice and liver damage.

Kemasin Semerak Sheep Farm (IADP), Pasir Puteh, Kelantan

In January 1984, 35 out of 160 local sheep in the newly established Kemasin Semerak Integrated Agriculture Development Project (IADP), Pasir Puteh, Kelantan died with signs of jaundice and photosensitization. These animals grazed on *B. decumbens* totally. The other affected sheep recovered once they were stallfed with *Setaria sphacelata* cv. *Kazungula*. No new cases were observed once feeding of *B. decumbens* was stopped (MEOR, *pers comm.*, 1984).

Other Incidences

Similar cases were seen in JOMIS sheep farm, Sungai Rengit Johore (DALZELL, *pers comm.*, 1982) and in Universiti Pertanian Malaysia goat farm, Serdang (ANDREW, *pers comm., 1983).*

RESEARCH FINDINGS IN MARDI

The incidences in the various farms suggest that there is a close association of this syndrome with grazing of *B. decumbens*.

An observation on 12 ISM grazing on *B. decumbens* showed that five of them died with signs of jaundice and photosensitization. There was marked increase in SGOT and total bilirubin values ranging from 330 – 1 891 unit/litre and 5.47 – 13.06 mg/100 ml respectively. The five carcasses showed ulceration on the skin particularly around the eyelids, muzzle, both upper and lower lips, tips of ear and vulva. There were also varying degrees of jaundice (Abas Mazni *et al.*, 1983).

Trials were conducted to determine the causative agent/s having recognized that total grazing of *B. decumbens* is the predisposing factor to liver damage in sheep. In another study, seven of the 12 ISM confined to a hectare of *B. decumbens* exhibited jaundice and photosensitization, an indication of liver damage nine days after grazing on pure stand of leafy regrowth 48 days after burning. Liver damage was confirmed by elevated levels of GGT (60 – 151 unit/litre) SGOT (250 – 742 unit/litre) and bilirubin values (Abas Mazni *et al.*, 1983). In another trial, 18 female ISM were randomly assigned to three treatment groups of six animals each. They were stallfed totally *B. decumbens* hay, green leafy *B. decumbens* or litter of this grass mixed with copra cake and mollasses *ad libitum*. The trial showed that ten of the 18 sheep showed varying degrees of jaundice as early as two weeks, five from the group fed on hay and five from those fed on green leafy *B. decumbens*. There was marked elevation of SGOT levels, varying from 208 to 628 unit/litre in five animals, two to four weeks after they fed hay while the values in those fed green grass were between 194 and 529 unit/litre, after the seventh week. Concurrently, the GGT values were between 107 and 255 unit/litre. None of the sheep fed on litter of this grass showed any obvious clinical signs. As shown by previous trials, no spore of *Pithomyces chartarum* was isolated. The trial indicated that total feeding of *B. decumbens* either in the form of green or hay caused liver damage and eventually jaundice. It was suggested that the unknown factor that was present in the plant could neither be destroyed nor its activity be reduced in the process of sun drying. It was hypothesized that the toxic agent could be in the plant itself with a direct mode of action or it could be in the form of its metabolites (Abas Mazni and Sharif, 1984).

Abas Mazni, Sharif, Mohd. Khusahry and Vance (1985) in a trial involving 12 goats grazing on *B. decumbens* continuously showed that the jaundice and photosensitization syndrome was observed in only three goats after 30 days of grazing. The clinical manifestations were similar to those observed in sheep grazing on this grass. The GGT levels in the affected goats were between 80 and 138 unit/litre, while the bilirubin values of these animals were between 5.4 and 13.7 mg/100 ml and a considerable fraction of total bilirubin was direct bilirubin, ranging from 71% to 90 per cent. Thus, goats on continuous grazing produced similar hepatotoxic syndrome but the effect was less severe.

In general, the findings from the trials conducted in MARDI are as follows:

a) Continuous grazing on pure stand of leafy regrowth of *B. decumbens* resulted in liver damage. Liver damage was confirmed by elevated levels of GGT, SGOT and bilirubin values.

b) Clinical signs observed were jaundice and photosensitization.

c) Necropsy indicated jaundiced carcass with slightly enlarged, firmer than normal liver and extended gall bladder.

d) Histopathology indicated hepatolobular necrosis suggesting that liver damage was of toxic in origin.

e) The saprophytic fungus, *P. chartarum* was not isolated and that the necropsy findings did not suggest Pithomycosis.

f) Haemolytic anaemia and haemoglobinuria were not a consistent finding in this toxicity.

g) This syndrome was not due to copper toxicity.

h) Goats on continuous grazing on *B. decumbens* produced similar hepatotoxic syndrome as in sheep. However, goats took a longer time to be affected and the effect was less severe.

i) The plant *B. decumbens* would cause hepatotoxicity to sheep and goats on total feeding.

j) Hay from *B. decumbens* would give a much faster hepatotoxic effect.

k) Recovered animals would be among the first to show the toxic effect if they were allowed to graze on this pasture again.

CLINICAL SIGNS

The clinical signs of jaundice and photosensitization were observed in sheep as early as nine days, ranging between nine and 60 days *(Plate 3)*.

On the onset of photosensitization, affected sheep preferred to stay under shade with both eyes closed by ocular lacrimation and conjuctivitis. Concurrently, jaundice was also observed. The yellow discolouration was usually observed on the conjunctiva, third eyelids and mucus membranes of the eye and vulva.

Following this, photosensitive dermatitis occurred. The skin lesions showed a characteristics distribution which were restricted to the unpigmented areas of the skin and those parts which were exposed to solar rays. Predilection sites for lesions were the ears, eyelids, muzzle, vulva and to lesser extent the perineum. The first sign was erythema, followed by oedema. Irritation in this area was intense and animals rubbed the affected parts often lacerating the face by rubbing it on the poles of the shed. The oedema was often severe and might cause drooping of the ears, and dysphagia due to swelling of the lips and tongue.

With prolonged exposure to sunlight the areas around the eyelids, lips, muzzle, ears and vulva became ulcerated. Exudation occurred especially around the eyelids and muzzle, and resulting in matting of the hair, and in severe cases, closure of the eyelids and nostril. Finally, it became thickened and leathery. As the condition of the sheep worsen due to progressive disturbance in the circulation of the subcutaneous tissue, the superficial layer of the skin degenerated and eventually sloughed off, leaving raw areas covered with dry exudates. The demarcation of lesion and normal skin was often very clear-cut particularly in animals with white patches. With the onset of symptoms of photosensitization, anorexia developed and the sheep would refuse to drink or eat resulting in dehydration and rapid loss of condition with marked decrease in body weight.

Nervous signs occurred in some animals and could be seen as head shaking, stamping of forelegs, dullness, incoordination and stargazing as accompaniments of liver dysfunction and jaundice.

Other signs observed were bilateral opacity, haemolytic anaemia and haemoglobinuria. This however was not a consistent finding. Only two out of 42 sheep showed these signs.

In goat, the days of onset of clinical signs ranged from 30 to 49 days. The clinical manifestations were similar to those observed in sheep grazing on *B. decumbens*. However, ulcerative dermatitis around the eyelids, muzzle, lips and ears were not as severe as those observed in sheep *(Plate 4)*. The signs were observed to be more severe in the Saanen and their crosses. Clinical signs were observed much earlier in sheep when compared to goats. The syndrome in sheep was observed within 20 days after grazing while in goat, the days of onset ranged from 30 to 49 days. Within a period of 60 days, more than 50% of the sheep grazing *B. decumbens* showed the clinical signs whereas in goat only 25 per cent. This indicated that, the effect of grazing *B. decumbens* was more severe in sheep than in goats.

CLINICAL PATHOLOGY

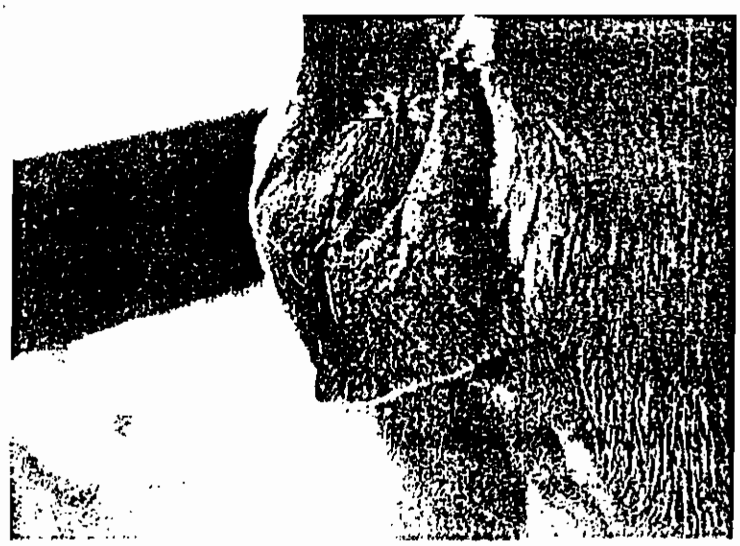
Haematological Pictures and plasma Protein

There were no significant changes in all these cases in the total erythrocyte count, haemoglobin concentration and packed cell volume (PCV) in sheep and goat. All these parameters appeared to be within the normal range even in those sheep and goat with liver lesions. In the experimental group, only two out of 42 sheep grazed on pure regrowth of *B. decumbens* showed haemolytic anaemia. Haemolysis was observed 12 and 22 days after grazing. In these animals the PCV decreased rapidly from 20% to 7.9% and 26% to 10% within two to four days. Haemoglobinuria was observed in both cases. These findings were inconsistent.

*Plate 4. Clinical signs of **Brachiaria decumbens** toxicity in goats.*

4a. Emaciated goat.

4b. Ocular lacrimation with swelling of the face, both eyelids and ears.



4c. Oedematous vulva. Blue discolouration is because of the application of fly repellants.

4d. Ulcerative dermatitis on the gum and lips.

The plasma protein increased gradually with increase in severity of jaundice and photosensitization, possibly because affected animals preferred to stay under the shed most of the times without eating or drinking, thus, causing dehydration. Furthermore, dysphagia occurred in animals due to the swollen tongue and lips.

Serum Glutamate Oxaloacetate Transaminase (SGOT) and Gamma-glutamyl Transferase (GGT)

Nearly all affected animals showing signs of jaundice and photosensitization showed a marked elevation of the SGOT and GGT enzyme levels. The increase ranged from 200 to 1 800 unit/litre and 107 to 345 unit/litre for SGOT and GGT respectively. Extremely high values of SGOT enzyme approaching 2 000 unit/litre, an increase of about 100 fold over normal values, were encountered in some sheep. SGOT values in normal sheep had been recorded by various authors and they ranged from 31 unit/litre (CORNELIUS, 1960) to 56 unit/litre (KUTTLER and MARBLE, 1958) and to 60-160 unit/litre (MALHERBE, 1960). The normal level of GGT is between 50 and 60 unit/litre. The increase indicated the presence of liver damage. In most cases, GGT was elevated first before any increase in SGOT. The elevation of GGT enzyme level was found to be a better indication of liver damage compared with SGOT.

Bilirubin

Jaundice cases were confirmed by icterus index and bilirubin values. Severe jaundice occurred in most of these animals with total bilirubin values ranging from 5.47 to 22.0 mg/100 millilitres. The normal bilirubin values are between 0 and 0.4 mg/100 ml (BLOOD, HENDERSON and RADOSTITS, 1979). In most of the animals, about 80% of the total bilirubin was conjugated bilirubin, indicating hepatocellular jaundice whereas in the two animals which had haemolytic anaemia, the indirect bilirubin values were much higher.

Serum, Liver and Kidney Copper Level

There was no marked increase in serum, liver and kidney copper levels in all the experimental animals. The copper levels in the liver and kidney ranged from 189.3 to 430.2 ppm, and 15.1 to 64.2 ppm, respectively. These are within the normal range. Results of copper analyses of the grasses showed that *Brachiaria* grass has the lowest copper level (4.65 ppm) compared with three other grasses namely *Digitaria setivalva*, (10.9 ppm) *Setaria sphacelata* var. *splendida* (11.2 ppm) and *Panicum maximum* (7.12 ppm).

GROSS AND HISTOPATHOLOGY

All affected carcasses showed ulceration on the skin particularly around the eyelids, lips, muzzle, ears and vulva indicating photosensitization. Varying degrees of icterus especially of the mucous and serous membranes were also observed *(Plate 5)*. Liver pathology was further confirmed by the presence of necropsy where evidence of liver changes were observed. The liver was consistently slightly enlarged, firmer than normal and had pale mottling. Gall bladder was consistently enlarged, containing markedly thickened bile *(Plate 6)*. There was no bile obstruction and no other significantly observable changes except in animals with haemolytic anaemia where the kidney was darker than normal.

Plate 5. Jaundiced carcass of sheep grazed on Brachiaria decumbens.

Plate 6. Mottling of liver with markedly distended gall bladder.

Most of the liver section indicated varying degree of hepatonecrosis, either diffuse or centrolobular. Most of which were acute hepatonecrosis. Cholangitis and mild bile retention were also observed in two animals. In the two animals with haemoglobinuria, a few tubules in the kidney were seen with haemoglobin cast, this is probably due to the haemolysis.

The specific causes of this syndrome remained unknown but the probable pathogenesis is described *(Figure 1)*. Findings indicated that the onset of this syndrome correlated with the ingestion of this grass, which could cause hepatotoxicity. When the liver was damaged, phylloerythrin, the metabolic by-product of chlorophyll and bilirubin, the red blood cell breakdown products were retained in the blood stream. Phylloerythrin, which was a photodynamic agent when activated by ultraviolet radiation of sunlight to sensitive tissue caused liberation of histamine, local cell death and tissue oedema. As the condition of the sheep worsened due to progressive disturbance in the circulation of the subcutaneous tissue, the superficial layer of the skin degenerated and eventually sloughed off, leaving raw areas covered with dry exudates. This manifested as ulcerative dermatitis of the exposed area on the muzzle, ears, lips and vulva as observed in the affected sheep. The circulating bilirubin caused the yellowish colour or jaundice as seen on the mucous membrane of the eyes and mouth. There was a variation in the hepatic lesions as seen by diffuse and centrolobular necrosis and cholangitis in few cases. In some of the cases, the lesion was seen only in parts of the liver. This was probably due to deposition of toxin in particular part of the liver due to portal streaming on its first passage through the hepatic sinusoids. Although in some sheep the liver was invariably damaged, indicated by marked elevation of GGT and SGOT, they failed to develop skin lesion and jaundice because the liver has considerable functional reserve. It must be severely damaged before its capacity to remove circulating photodynamic agent or bilirubin was significantly impaired. It appeared that at the initial stages of liver damage, corresponding changes in weight began to appear. However, as liver damage became more pronounced, weight was significantly altered. The decrease in weight of sheep with liver damage was probably due to nutritional stress. This might have been a simple reduction of intake since severely affected animals were normally observed to have abandoned grazing or it might have been due to lowered capacity to utilize ingested feed (CAMPBELL and WESSELINK, 1973). As soon as liver damage was repaired weight gain returned to normal.

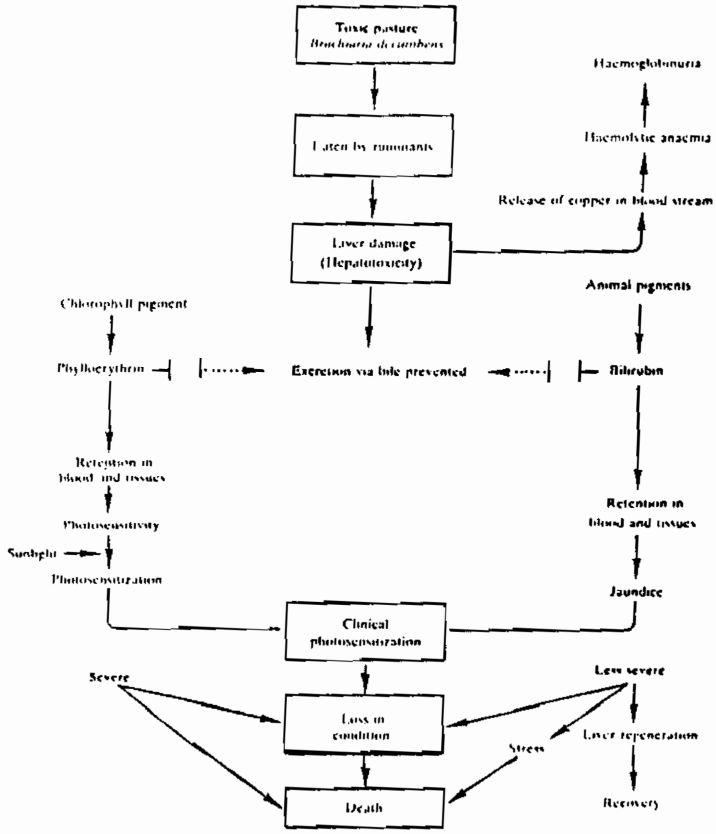


Figure 1. The probable pathogenesis of Brachiaria decumbens toxicity.

DISCUSSION

There is a similarity in the clinical signs of photosensitivity in sheep grazing *B. decumbens* with those of facial eczema which is caused by the saprophytic fungus, *Pithomyces chartarum* (Berk and Curt) M.B. Ellis as described in New Zealand (CUNNINGHAM, HOPKIRK and FILMER, 1942), in Australia (HORE, 1960) and in South Africa (MARASAS, ADELAAR, KELLERMAN, MINNE, VAN RENSBURG and BORROUGHS, 1972). Facial eczema is caused by ingestion of spores of *P. chartarum* containing the hepatotoxic mycotoxin sporidesmin (BROOK and WHITE, 1966; TAYLOR, 1967; CEIEGLER and LILLEHOJ, 1968; WRIGHT, 1968; BROOK, 1969). Similar observations were made in sheep and cattle in tropical areas of Colombia and Brazil, South America.

Several workers have suggested this saprophytic fungus as the primary etiological entity, however the primary cause of the syndrome has not been clearly defined (CARMAGO *et al.*, 1976; NOBRE and ANDRADE, 1976; DOBEREINER *et al.*, 1976; ANDRADE *et al.*, 1978; OLIVEIRA *et al.*, 1979). It was suggested that the nature of growth of *B. decumbens* provided a good substrate for the fungus to grow. However, the level of the fungus or its spore on the pasture in these areas was found to be low, one to three spores per gramme of leaf dry weight (ANON., 1980). Furthermore, the concentration of toxin extracted from the fungus that was isolated in Colombia was much lower than that in Australia and New Zealand. In New Zealand, Australia and Uruguay there was a direct correlation between number of spores on the pasture and presence of the animals with symptoms (RIET ALVARIZA, PERDOMO, CORBO, BERIAO, DE LA PENA, PARADA, MICHELENA, QUADRELLI and ALZUGARAY, 1977). For instance in New Zealand, facial eczema cases occurred only when spore counts on the grass were over 10^5 per gramme of leaf dry weight (BROOK, 1969). Workers in South America had not found similar correlation. Hence, it was concluded that other factors might be involved in the etiology of the syndrome (ANON., 1981). Concurrently there has been several groups that have tried to incriminate the plant, *B. decumbens* as to cause the toxicity directly, however, the evidence is meager (AYCARDI, *pers comm.*, 1983).

No isolation of this saprophytic fungus was made from the grass and suspected pasture litter in our studies. Fungal isolation techniques used were as those recommended by the Ministry of Agriculture and Fishery, New Zealand (CHAPMAN and DI MENNA, 1981) and THORNTON AND SINCLAIR (1960). However, there were *Cladosporium, Penicillium, Nigrospora* and *Spegazzinia* spp.

If P. chartarum is the etiological agent that causes the syndrome, then the process of burning, removal of pasture litter (substrate for fungal growth) and disc ploughing would have minimized the presence of fungus or its spores. Surprisingly, sheep grazed on pure leafy regrowth of this pasture after the above processes were carried out, showed a much faster effect (ABAS MAZNI et al., 1983). Similar group of sheep grazing on adjacent plot of other grasses under exactly the same management were not affected. The feeding trial where sheep were stallfed green, hay and litter of this pasture further supported the elimination of this fungus involvement. None of the sheep fed with litter of this grass which is a substrate for fungal growth, showed any indication of liver damage. Whereas, sheep fed green and hay of *B. decumbens* showed marked elevations of SGOT and GGT enzyme levels, with varying degrees of jaundice and photosensitization (ABAS MAZNI and SHARIF, 1984).

The necropsy findings of *B. decumbens* toxicity in sheep and goat do not conform to that of facial eczema (Pithomycosis), thus not suggestive of sporidesmin poisoning. In sporidesmin poisoning the essential lesion is an acute cholangitis which progresses to obliteration of bile ducts by fibrous tissues leading to ductual hyperplasia, canalicular infarction and hepatocellular necrosis. Hepatocyte injury was not marked and developed largely as a consequence of bile duct occlusion and biliary stasis, *i.e.* it was largely a secondary change in hepatocytes (McFARLANE, EVANS and REID, 1959; MORTIMER, *pers comm.*, 1983). In sheep and goats fed with *B. decumbens*, the hepatocyte lesion appeared to be the primary change and biliary stasis probably emanated from a lesion of the hepatocyte/canalicular interface or from bilirubin/phylloerythrin conjugation failure or failure of transport across the hepatocyte (MORTIMER, *pers comm.*, 1983). Sections of liver of all affected sheep showed a consistent picture of diffuse hepatocellular necrosis lesion. There was little or no reaction in portal tracts and no evidence of bile duct injury. With the above findings the possibility of fungal involvement is eliminated.

Liver damage which was shown by marked elevation of GGT and SGOT was consistently observed in all the affected animals. Jaundice which is secondary to the liver damage was indicated by high bilirubin values. VAN den BERGH test indicated that a considerable fraction of the total bilirubin was conjugated or indirect bilirubin. Thus, hyperbilirubinemia is due to hepatic or obstructive jaundice. Ulceration due to photosensitization and emaciation can also give rise to an increase in SGOT level. In some cases, animals not showing clinical signs with low SGOT level showed obvious increase in GGT level, indicating liver damage. The liver damage in these animals was not accompanied by clinical signs of jaundice and photosensitization probably due to liver functional reserve. This is clearly observed in goats where liver damage occurred without obvious clinical signs. Elevation of GGT level was found to be a better indication of liver damage compared to SGOT. Both SGOT and GGT enzyme levels decrease once an affected animal is removed from *B. decumbens* pasture.

Haemolytic anaemia and haemoglobinuria were observed in a small proportion of the animals with clinical signs in the field incidences. In this study, only two out of 42 sheep and 12 goats showed the above signs. This is not a consistent finding in *Brachiaria* toxicity and was thought to be due to copper toxicity. In our findings, there was no increase in the serum copper level in all the affected animals even in the two animals with haemolytic anaemia and haemoglobinuria. The liver and kidney copper levels of all the affected sheep including those with haemolytic anaemia were within the normal range (less than 400 ppm for liver). It is impossible to define normal values for levels of copper in the liver, but for present purposes it is a reasonable generalization that levels up to 500 ppm dry weight pose no threat of untoward effect. In levels increasing above 800 ppm, there is a corresponding increase in the liability to sudden release and haemolysis (JUBB and KENNEDY, 1970). For instance, in chronic copper poisoning, the copper level in the liver of Nubian goats ranged from 823 to 2 054 ppm (ADAM and WASFI, 1977). Furthermore, in our studies the animals were fed solely on *B. decumbens* with no concentrate and mineral supplement. The copper level in the *Brachiaria* was 4.65 ppm, which is much lower when compared to other pasture grass such as *Digitaria, Setaria* and *Guinea.* These grasses have copper values ranging from 9.0 to 11.0 ppm, (CHEN, AJIT and EVANS 1981; ABAS MAZNI et. al., 1983). Necropsy and histopathology of kidneys of affected sheep and goats did not indicate sign of toxic nephrosis as in copper poisoning. Hence our findings indicate that copper is not the primary cause of the syndrome. If it plays a part as observed in copper toxicity due to hepatotoxic plants, it only plays a secondary role to the liver damage. In sheep, this is a common phenomenon since sheep accumulate copper in the liver and sudden release of this mineral occurred when the liver is damaged. The haemolytic anaemia and haemoglobinuria which sometimes occur in this *Brachiaria* intoxication is probably not due to copper but some other causes.

No other plants except for few legumes, *Centrosema pubescens* and *Calopogonium mucunoides* were found growing in the *B. decumbens* pasture plot. The possibility of another primary plant causing the photosensitization is therefore eliminated.

CONCLUSION

Evidence of the incidences in the field and research findings indicated that the jaundice and photosensitization syndrome observed in the sheep and goats grazing on *Brachiaria decumbens* was due to liver damage (hepatolobular necrosis). The *B. decumbens* toxicity causes acute hepatic necrosis resulting in hepatic insufficiency manifested by loss of condition, hepatic jaundice, photosensitization and death. Liver damage occurred much earlier when animals were fed hay compared with those fed green Brachiaria. Thus total feeding of *B. decumbens* either in the form of green grass or dry hay is not advisable for sheep and goats.

RECOMMENDATIONS AND RESEARCH NEEDS

There is no specific treatment for *B. decumbens* toxicity. Only the secondary symptoms can be treated while the sheep or goats liver heals itself. All affected animals should be removed from the pasture or hay into the shade away from direct sunlight and fed with high quality feed. Preventive measures should be undertaken against secondary infection especially maggot infestations on the ulcerative lesion as a result of photosensitization on the skin. Skin lesion may recur if the liver is still damaged on release. Animals that have recovered will be among the earliest to show the syndrome as soon as they are allowed to graze on this pasture. Therefore sheep which apparently recovered should be culled when saleable as they will be susceptible to subsequent stresses. The farmers should be aware of the disease and at the appearance of symptoms follow necessary recommendations to avoid a higher number of animals being affected.

It is not wise to eliminate this grass as it is highly productive and valuable in the tropical areas. Furthermore this grass has been extensively planted in our country. Utilization of this grass as a total feed to sheep and goats should be avoided. Since this syndrome also occurred sporadically in cattle as reported in other countries, we should be aware of its occurrence. In order to be prepared against possible and unexpected occurrences in cattle, it is necessary to rotationally graze the animals on different forages.

Research should be conducted to determine methods of utilizing this pasture so as to prevent and minimize the adverse effect. Since there is an indication of different breed susceptible to *Brachiaria* toxicity in goat, further studies should be carried out.

However, a number of questions still remain unanswered particularly the question of the hepatotoxicity of the unknown hepatotoxic factor. The toxic agent could be in the plant itself and acts directly or it can be in the form of its metabolites. Further work is needed to identify the toxic principle and their mode of actions.

ACKNOWLEDGEMENTS

The authors wish to thank Tuan Syed Ali Syed Abu Bakar, former Director of Livestock Research Division, Mr. Ahmad Tajuddin Zainuddin and Mr. Mohd. Khusahry Mohd. Yusoff for their encouragement and support. They gratefully acknowledge the advice and assistance of Dr. Margaret di Menna and Dr. Peter H. Mortimer of the Ruakura Animal Research Station, Hamilton, New Zealand; Mr. Anthony Johnston of the Commonwealth Mycological Institute, UK; Mr. J.P. Langlands of the Pastoral Research Laboratory CSIRO, Australia; Mr. E.F. Henzell of the Division of Tropical Crops and Pastures, CSIRO, Australia; Mr. L.L. Callow of the Animal Research Institute, Brisbane, Australia; Mr. B.A. Woolcock of the Division of Animal Industry, Dept. of Primary Industries Brisbane; Mr. J.M. Hopkinson of the Research Station Walkamin, Australia; Mr. Carlos Lascano of the Centro Internasional de Agricultura Tropical, Cali, Colombia; Mr. D.S. Loch of Dept. of Primary Industries, Gympie, Australia; Mr. B. Eduardo Aycardi B. of the Empresa Colombiana de Productos Veterinarious, Bogotà, Colombia; Dr. A.R. Sheikh Omar, Dr. H.N. Vance and Dr. Mohd. Zamri Saad of the Dept. of Pathology, Universiti Pertanian Malaysia; Dr. Andrew Alex Tuen of Dept. of Animal Science, Universiti Pertanian Malaysia; Mr. Hanifah Abu Bakar; Mr. Abdul Manaf Mohd. Kasim; Mr. Che Aziz Mohamood; Mr. Chen Chin Peng; Dr. Dahlan Ismail; Mr. R. Dalzell, of JOMIS; Dr. Lim Bee Guan; Mr. Meor Hashim; Mr. Tuan Ngah Tuan Baru; Mr. Kamaluddin Majid; Ms. Roshidah Mutalib; Mr. Kamaruzaman Mohamad; Mr. Malik Abdullah; Staff of the Goat and Sheep Unit, MARDI and those who helped and supported the research in one way or another.

ABSTRACT

Jaundice and photosensitization syndrome is a hepatotoxic disease of ruminants caused by total feeding of *Brachiaria decumbens*. It is characterized mainly by toxic liver damage (hepatolobular necrosis) which leads to weight loss, jaundice, photosensitization and death. Incidences of the disease were reported in cattle and sheep in Colombia and Brazil, South America, while in Malaysia it occurred mainly in sheep and goats. The toxicity is remarkably potent for sheep and goats causing a mortality rate of up to 50% while in cattle clinical cases appear in only 4.7% of affected cattle. Cattle between nine and 24 months are especially involved and subclinical toxicity is also observed bringing about loss of body condition. While the actual toxic principle remains unknown, the circumstances of poisoning and the pathogenesis of the disease are becoming clearer.

This paper reports the incidences and research conducted on jaundice and photosensitization syndrome due to hepatotoxicity related to total feeding of *B. decumbens*. The clinical signs, clinical pathology, postmortem and histopathology are also described.

REFERENCES

ABAS MAZNI, O., MOHD. KHUSAIRY, M.Y. and SHEIKH OMAR, A.R. (1983). Jaundice and photosensitization in Indigenous Sheep of Malaysia grazing on *Brachiaria decumbens*. *Mal. Vet. J.* *7*, 254–63.

ABAS MAZNI, O. and SHARIF, H. (1983). Laporan kajian penyakit dan kematian kambing dan biri-biri di Pusat Pembiakan Kambing, Gajah Mati, Kedah, 9 pp.

——— (1984). Hepatotoxicity in Indigenous Sheep of Malaysia stall-fed different forms of *Brachiaria decumbens*. *(unpubl.)*

ABAS MAZNI, O., SHARIF, H., MOHD. KHUSAIRY, M.Y. and MOHD. ZAMRI, S. (1983). Hepatogenous photosensitivity in Indigenous Sheep of Malaysia grazing on pure stand of leafy regrowth of *Brachiaria decumbens*. *Mal. Vet. J.* 7, 271.

ABAS MAZNI, O., SHARIF, H., MOHD. KHUSAIRY, M.Y. and VANCE, H.N. (1985). Photosensitization in goats grazed on *Brachiaria decumbens*. *MARDI Res. Bull.* *13*(2), 203–6.

ADAM, S.E.I. and WASFI, I.A. (1977). Chronic copper toxicity in Nubian goats. *J. Comp. Path.* *87*, 623–7.

ANDRADE S.O., LOPES, H.O. da SILVA, BARROS, M.A., LEITE, G.G., DIAS, S.M.C., SAUERESSIG, M., NOBRE, D. and TEMPERINT, J.A. (1978). Aspects of photosensitization in bovines grazing on pasture of *Brachiaria decumbens* Stapf. infested with *Pithomyces chartarum*. *Arq. Inst. Biol Sao Paulo* *45*, 117–36.

ANON. (1978). Tropical Pasture Programs, *Ann. Rep.*, p. 148. Cali, Colombia:CIAT.

——— (1979). Tropical Pasture Programs, *Ann. Rep.*, pp. 113–4. Cali, Colombia: CIAT.

——— (1980). Tropical Pasture Programs, *Ann. Rep.*, p. 110. Cali, Colombia: CIAT.

——— (1981). Tropical Pasture Programs, *Ann. Rep.*, pp. 278–82. Cali, Colombia: CIAT.

——— (1982). Tropical Pasture Programs, *Ann. Rep.*, pp. 9–20. Cali. Colombia: CIAT.

BLASCO, L.M. and BOHOROUEZ, A.N. (1967). Composicion quimica de plantas forrajeras (gramineas) comunes en el valle del Cauca. *Acta agron., Palmira* *17*, 13–9.

BLOOD, D.C., HENDERSON, J.A. and RADOSTITS, O.M. (1979). *Veterinary Medicine* 2nd ed. London: ELBS and Balliere Tindall.

BRITON, N.W. and PALTRIDGE, T.B. (1940). Preliminary note on photosensitization on sheep grazing on *Brachiaria brizantha*. *Royal Society of Queensland. Proc.* *52*, 121–2.

BROOK, P.J. (1969). *Pithomyces chartarum* in pasture and measures for the prevention of facial eczema. *J. Stored Prod Res.* *5*, 203–9.

BROOK, P.J. and WHITE, E.P. (1966). Fungus toxins affecting mammals, *A. Rev. Phytopath.* *4*, 171–94.

CAMPBELL, A.G. and WESSELINK, C. (1973). Facial eczema, liver damage and liveweight change in lambs. *N.Z. J. Expt. Agric.* *1*, 291.

CARMAGO, V.A. W., WALTER, N., NELSON, S. F. and REGINA, E.M.A. (1976). Photosensitization in calves grazing herbage of Brachiaria decumbens *O. Biologico Sao Paulo* *42*, 259–61.

CATTORD, J.R. (1952). Pasture management in Uganda in relation to Western Equatoria. *E. Afr. agric. J.* *17*, 183–7.

CEIFOLFR, A. and LILLEHOJ, E.S. (1968). Mycotoxins. *Adv. appl. microbial* *10*, 155–219.

CHAPMAN, R. and DI MENNA, M.E. (1981). Facial eczema predicting danger periods by spore counting. Farm production and practice. Ministry of Agriculture and Fisheries, New Zealand. *Aglink 3/6000/5/81 : EPP 494.*

CHEN, C.P., AHI, S.S. and EVANS, T.R. (1981). Beef production from three nitrogen fertilized grasses in Peninsular Malaysia. *MARDI Res. Bull.* *9*, 207–24.

CLARE, N.T. (1952). Photosensitivity in diseases of domestic animals. Commonwealth Bureau Animals Health Revue Series 3.

CORNELIUS, C.E. (1960). Serum transaminase in veterinary diagnostic. *Calif. Vet.* *13*, 22– 3.

CUNNINGHAM, I.J., HOPKIRK, C.S.M. and FILMER, J.F. (1942). Photosensitivity disease in New Zealand. 1. Facial eczema. Its clinical, pathological and biochemical characteristics. *N.Z.J. Sci. Technol.* *24*, 185–98.

Dobereiner, J., Tokarnia, C.H., Monteiro, M., Cruz, L.C.H., Carvalho, E.G. and Primo, A.T. (1976). Intoxication of cattle and sheep in pasture of *Brachiaria decumbens* contaminated with *Pithomyces chartarum*. *Pesquisa Agropecuaria Brasileira, Serie Veterinaria 11*, 87–94.

Dougall, H.W and Bogdan, A.V. (1958). The chemical composition of the grasses of Kenya. *E. Afr. agric. J. 24*, 17–21.

Garcia, O., Aycardi, E., Zuluaga, F.N., Rivers, B. and Henao, F.J. (1982). Aspectos epidemiologicos de la fotosensibilizacion hepatotoxica asociada al pastoreo de Brachiaria decumbens en los Lalanos Orientales de Colombia. *Revta Acovez 6*, 5–11

Hort, D.E. (1960). Facial eczema. *Aust. Vet. J. 36*, 172–6.

Jubb, K.V F. and Kennedy, P.C. (1970). *Pathology of Domestic Animals* 2nd ed., p. 305. New York: Academic Press.

Judd, B.I (1975) New world tropical forage grasses and their management. 6. Bahiagrass, Rivergrass, Signal grass. *Wld Crops* 27, 175–7.

Kuttler, K.L. and Marble, D.W. (1958). Relationship of serum transaminase to naturally occurring and artificially induced white muscle disease in calves and lambs. *Am. J. Vet. Res. 19*, 623–36.

Loch, D.S. (1977). *Brachiaria decumbens* (signal grass) – A review with particular reference to Australia. *Tropical Grasslands 11*, 141–57.

Mackay, J.H.E. (1974). Register of Australia herbage plant cultivars. A. Grasses. 16. Brachiaria a *Brachiaria decumbens Stapf. (Signal grass) cv. Basilisk (Reg. No. A – 16a – 1). J. Aust. Inst. agric. Sci. 40*, 91–93.

Malherbe, W.D. (1960). *J.S. Afr. vet med. Ass. 31*, 159. Cited by Done J., Mortimer, P.H. and Taylor, A. (1962) in *Res. Vet. Sci. 3*, 161–71.

Marasas, W.F.O., Adelaar, T.F., Kellerman, T.S., Minne, J.A., Van Rensburg, I.B.J. and Borroughs, G.W. (1972). First report of facial eczema in sheep in South Africa. *Ondersteport J. Vet. Res. 39*, 107–12.

McFarlane, D., Evans, J.V. and Reid, C.S.W. (1959). Photosensitivity diseases in New Zealand. XIV. The pathogenesis of facial eczema, *N.Z.J. Agric. Res. 2*, 194–200.

Miller, I.L. (1974). Plant collection in Mexico, Venezuela and Colombia for the high rainfall zone of the Northern Territory. *Plant Introduction Review 10*, 13–9.

Nobre, D. and Andrade, S.O. (1976). Relationship between bovine photosensitization and the grass *Brachiaria decumbens* Stapf. *Biologico 42*, 249–58.

Oliveira, G.P., Novaes, A.D., Costa, A.J. and Andrade, O. (1979). Photosensitization in sheep caused by *Pithomyces chartarum* in pasture of *Brachiaria decumbens* in Sao Carlos Region, Sao Paulo State. *Cientifica 7*, 17–22.

Rattray, J.M. (1960). The grass cover of Africa, F.A.O Agricultural Studies No. 49, Food and Agriculture Organisation, Italy.

Richards, J.A. (1970). Productivity of tropical pastures in the Caribbean. Proceedings of the XI International Grassland Congress. Australia. pp. 49–56.

Riet Alvariza, F., Perdomo, E., Corbo, M., Beriao, J., De La Pena, L., Parada, L., Michelena, E., Quadrelli, J.C. and Alzugaray, I. (1977). Nuevos casos de fotosensibilizacion hepatogrena. Producidos por el hongo de la pradera, *Pithomyces chartarum* (Bert and Curt) M.B. Ellis. Documento Interno, CIVET., Miguel, C. Rubino, Casilla de Correo, 1977, Monte video, Uruguay.

Santhirasegaram, K., Diez, J., Petersen, N.F. and Trigueros, M.V. (1974). Milk production from four tropical pasture systems. Proceedings of the XII International Grassland Congress, U.S.S.R. Sectional Paper, Grassland Utilization II, pp. 599–605.

Shahirudin S., Tan, S.L. and Rajendram, S. (1983). Cases of jaundice associated with copper poisoning in goats in Pusat Ternakan Haiwan, Batu Arang. *Paper presented at 11th Annual Conference AVSM, Kuala Lumpur, 1983*

Sturtz, J D., Harrison, P.G. and Falvey, L. (1975). Regional pasture development and associated problems. II. Northern Territory. *Tropical Grassland, 9*, 83–91.

Suparjo, N.M. and Abdul Wahid, S. (1980). Pengenalan awal pada masalah memberi rumput jenis *Brachiaria decumbens* kepada kambing dan biri. *Paper presented at 25th Annual* Conference MVA, Kuala Lumpur, 1980.

TAYLOR, A. (1967) The chemistry and biochemistry of sporidesmins and other 2, 5 − epidithio − 3, 6, dexopipera − zines. In *Biochemistry of Some Food Born Microbial Toxins.* (ed. MATELES, R.I. and WOGAN, G.N.) Cambridge, Massachusetts: M.I.T. Press.

TERGAS, L.E., PALADINES, O. and KLEINHEISTERKAMP, I. (1983). Animal productivity and pasture management of *Brachiaria decumbens* Stapf. in Colombian Llanos. *Tropical animal production* *8* (in press).

THORNTON, R.H. and SINCLAIR, D.P. (1960). Some observations on the occurrence of Sporidesmin bakeri Syd. and facial eczema disease in the field. *N.Z. J. Agric. Res.* *3*, 300−13.

TRIBUDHI, M., HELMY, H., VAN EYS, J., WILSON, A.J., PADERI, Z. and STOLZ, D.R. (1983). Studi pendahuluan kasus keracunan *Brachiaria sp. Paper presented at Seminar Ruminansia Kecil, Bogor, Indonesia, 1983.*

WINTER, W.H. (1976). Preliminary evaluation of twelve tropical grasses with legumes in northern Cope York Peninsular. *Tropical Grassland* *10*, 15−20.

WONG, C.C. (1980). Productivity and chemical composition of 20 improved tropical grasses in the humid tropics. *MARDI Res. Bull.* *8*, 163−73.

WRIGHT, D.E. (1968). Toxins produced by fungi. *A Rev. Microbiol.* *22*, 269−82.

that ill health was the cause. Work clearly precipitated the situation as the buffaloes became progressively weaker over the three days work. It is suggested that the decline in work output was associated with a parasitic or disease infection. Further studies will be carried out to verify and quantify these effects.

ACKNOWLEDGEMENT

The author is grateful to Dr P. R. Lawrence, C. Ghimire, N. Gurung and R. F. Archibald for their assistance in various aspects of this study.

Accepted for publication December 1988

REFERENCES

LAWRENCE, P. R. (1985). A review of the nutrient requirements of draught oxen. In: Draught Animal Power for Production, ACIAR Proceedings Series No. 10. ACIAR, Canberra, pp 59–63.

PEARSON, R. A. (1989). *Animal Production*, 49, (in press).

PEARSON, R. A., LAWRENCE, P. R. & GHIMIRE, C. (1989). *Animal Production* 49, (in press).

RUKMANA, M. P. (1979). Microhaematocrit method as a new technology in diagnosing surra and its relevancy to livestock socioeconomics. PhD Thesis. University Padjadaran, Bandung, Indonesia.

BOOK REVIEW

World Dictionary of Livestock Breeds. 3rd edn. I. L. Mason. C·A·B International, Wallingford. 1988. 348 pp. £23.63 UK. £25.95 elsewhere. US$47.50 Americas. ISBN 0-85198-617-X.

The third edition of this classic book of reference contains 3,000 new entries and revisions of many of the old ones. The dictionary lists names and salient physical features of breeds, types and common commercial crosses of cattle, sheep, horses, pigs, goats, buffaloes and asses throughout the world; there are also references to Breed Societies and Handbooks. The book is wholely a work of reference and is recommended for purchase by libraries rather than by individuals.

It is difficult to keep a compilation of this nature up-to-date and the reviewer noted some omissions of developments occurring during the past 15 years in tropical breeds he has been involved with: eg. no mention of the admix of *Bos taurus* (Friesian) blood to the Mpwapwa during the mid-seventies. However, these are criticisms of minor importance and the book remains a standard, authoritative reference work on livestock breed nomenclature throughout the world.

J. S. Macfarlane

Trop Anim Hlth Prod (1989) 21, 277–280

Short Communication

PHOTOSENSITISATION: A NOTE OF CAUTION IN THE USE OF *BRACHIARIA* PASTURES—A REVIEW

R. BABER[1]

Animal Production and Health Division, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy

Photosensitisation in ruminants is not a recently discovered problem and reference to facial eczema, which is a form of photosensitisation, was made at the end of the nineteenth century. Since then a considerable amount of research has been carried out on photosensitisation, particularly at the Ruakura Animal Research Station, New Zealand. A comprehensive review of hepatogenous photosensitivity diseases which covers not only the principal causes of photosensitivity but also its diagnosis has been made by Kellerman and Coetzer (1984).

The cause of photosensitisation can be primary or secondary. A primary agent is normally a photodynamic pigment which is absorbed from the gastro-intestinal tract, enters the peripheral circulation via an unimpaired liver and reacts with light at the surface of the skin. Secondary or hepatogenous photosensitisation occurs indirectly as a result of damage to the liver. When this occurs phylloerythrin, a break-down product of chlorophyll produced by gut micro-organisms, is retained and enters the peripheral circulation. When phylloerythrin comes into contact with sunlight in exposed unpigmented parts of the body it causes oxidative changes in the cells of the skin and adjacent tissues. Visible signs include the tendency by affected animals to avoid direct sunlight, pruritis, erythema and swelling of the affected parts; this is then followed by loss of skin and potentially, death.

Primary photosensitisation is not as common as the secondary form. Kingsbury (1964), Kellermann and Coetzer (1984) and Cheeke and Shull (1985) mention that fagopyrin, hypericin and furanocoumarins, which are found in such common plants as *Fagopyrum sagittatum* (buckwheat), *Hypericum perforatum* (St Johns wort), *Ammi majus* (bishops weed) and *Cymopterus watsonu* (spring parsley) respectively, are all primary photosensitising agents. Secondary photosensitisation is caused by a wide variety of different plants as well as by two fungi and by an algae (Kellerman and Coetzer, 1984). Of the plants that cause secondary photosensitisation, *Lantana camara* is probably the most ubiquitous. However, Kingsbury (1964), Kellerman and Coetzer (1984) and Cheeke and Shull (1985) mention other plants such as *Tetradymia* spp. (horsebrush) and *Tribulus terrestris* which are also important. Of more immediate concern, however, is photosensitisation caused as a result of the ingestion of toxins produced by fungi that are associated with pastures. Brook (1969) pointed out that facial eczema in New Zealand is caused as the result of the ingestion of the toxin sporidesmin produced by the saprophytic fungus, *Pithomyces chartarum*, associated with pastures.

During the past five to 10 years there has been a large increase in the use of sown pastures in the developing countries. In Latin America as well as in Africa and Asia, the *Brachiaria* group of grasses has proved to be very successful. *B. decumbens* in particular, as a result of its tolerance to low fertility and acid soils,

[1] Present address: Reed Farm, Christow, Exeter EX6 7NR, UK.

ease of establishment, aggressive growth habit and high productivity, has become widely used. However, there is increasing evidence to suggest that caution should be exercised in the use of *Brachiaria* pastures. In Asia Othman, Yusuf and Abdul Rahman (1983) reported 41% mortality and 100% morbidity in a group of 12 ewes grazing *B. decumbens* in Malaysia, while Murdiati and Lowry (1983) noted problems of photosensitisation in sheep grazing or being fed silage from *Brachiaria* pastures in Indonesia. In Africa Pierre (1984) reported an outbreak of photosensitisation in the Ivory Coast in 20 flocks of sheep grazing mixed pastures including *B. ruziziensis*. The problem was overcome by replacing *Brachiaria* with *Panicum* or *Chloris*.

In South America there are various reports of the problem of photosensitisation in association with *Brachiaria* pastures, only some of which are reported here. CIAT (1984) reported 32% morbidity and 27% mortality in a control group of cattle grazing a *B. decumbens* pasture in a trial to determine the efficacy of zinc in the control of photosensitisation. Garcia, Aycardi, Zuluaga, Rivera and Henao (1982) reported 4·7% morbidity and 2·7% mortality in 961 cattle grazing *B. decumbens* and 13% mortality in a group of 123 lambs of three to five months of age. Temperini and Barros (1977) in a review of the problem of photosensitisation mentioned its importance in young cattle grazing *B. decumbens* pastures. Tokarnia, Dobereiner and da Silva (1979) stated that three species of *Brachiaria*, namely *B. radicans*, *B. decumbens* and *B. brizantha*, are known to cause photosensitisation in cattle in Brazil. Garcia (pers. comm.) commented that in Brazil problems of photosensitisation have also occurred on *B. humidicola* pastures. Various workers have reported the presence of *P. chartarum* in *Brachiaria* pastures (Lau and Singh, 1985; CIAT, 1984; Tokarnia, *et al.*, 1979). CIAT (1984) also reported the presence of *P. chartarum* in the legume *P. phaseoloides* grown in association with *B. decumbens*.

The aforementioned observations clearly show that many of the *Brachiaria* grasses can be associated with the problem of photosensitisation. It should not be thought that they are the only grasses involved as the problem in New Zealand occurs on predominantly *Lolium perenne* swards and Dollahite, Younger and Jones (1977) and Muchiri, Bridges, Veckert, and Bailey (1980) reported the problem on *Panicum coloratum* pastures. It therefore needs to be firmly established whether the risk of photosensitisation is greater on *Brachiaria* pastures than it is on other pastures.

In consideration of the potential that the *Brachiaria* spp. offer to animal production this issue deserves further attention. While it seems likely that *P. chartarum* is an important agent in causing photosensitisation in animals grazing *Brachiaria* pasture, it is not known if it is the only agent. Othman, Haron, Yusuff and Saad (1983) were unable to isolate spores of *P. chartarum* from a *Brachiaria* pasture which had caused problems of photosensitisation and Murdiati and Lowry (1983) on the basis of experience in New Zealand and a personal communication with P. Mortimer and M. di Menna of the Ruakura Animal Research Centre, New Zealand, question whether the photosensitisation they found on *Brachiaria* pastures is due to *P. chartarum*. They commented that the chemical properties of sporidesmin would not suggest that this toxin is responsible for photosensitisation in tropical cases and that data from South America do not conclusively show that the problem encountered there is facial eczema.

Outbreaks of photosensitisation are sporadic and considerable areas of *Brachiaria* pastures are being grazed without any problem; the reason for this has

not as yet been determined. While the most dramatic effect of photosensitisation, death, is often reported there is far less information on its effect on the liveweight gain of animals grazing these pastures. This latter question is potentially far more serious and CIAT (1984) reported weight losses of 1·2 kg/day in cattle that subsequently develop clinical signs of photosensitisation.

There are many issues which need to be clarified because reaction to the problem of photosensitisation in animals grazing *Brachiaria* pastures has often been one of two extremes. In some countries *Brachiaria* spp. have been almost completely disregarded due to fears about photosensitisation, whilst in others *Brachiaria* is used without any knowledge of this potential problem. Action based on fact is required and it is suggested that the following key questions should be answered:

1. Is the prevalence of photosensitisation on *Brachiaria* pastures greater than that on other pastures?
2. Can the problem of photosensitisation be reduced by planting mixed pastures or by adopting different pasture management practices?
3. Are we sure that the problem is due only to the toxin sporidesmin produced by the fungus *P. chartarum*; the presence of a co-factor would explain the sporadic occurrence of outbreaks of photosensitisation. Indeed is the problem itself caused by a weed or other plant commonly found in association with *Brachiaria* pastures?
4. Is there any difference in animal species' susceptibility to photosensitisation?
5. What is the real prevalence rate of photosensitisation?
6. How serious is sub-clinical photosensitisation on *Brachiaria* pastures which results in reduced weight gains and is this the reason for the general disappointing level of animal performance on many tropical pastures?

Answers to these questions would allow a more rational assessment of the risk to be encountered in the use of *Brachiaria* pastures. It is particularly important to decide if the problem of photosensitisation is one to be lived with, in which case control measures such as those used in New Zealand and described by Brook (1969) are required, or whether it is a problem to avoid by not growing *Brachiaria*.

Accepted for publication October 1988

REFERENCES

BROOK, P. J. (1969). *Journal of Stored Products Research*, 5, 203–209.

CHEEKE, P. R. & SHULL, L. R. (1985). Natural toxicants in feeds and poisonous plants. AVI Publishing Co, Westport, Connecticut, USA. pp 456–460.

CIAT (1984). Annual Report 1984. Tropical pastures program. Centro Internacional de Agricultura Tropical.

DOLLAHITE, J. N., YOUNGER, R. L. & JONES, L. P. (1977). *Journal of the American Veterinary Medical Association*, 171, 1264–1265.

GARCIA, O., AYCARDI, E. R., ZULUAGA, F. N., RIVERA, B. & HENAO, F. J. (1982). *ACOVEZ*, 6(21), 5–11.

KELLERMAN, T. S. & COETZER, J. A. W. (1984). Hepatogenous photosensitivity diseases in South Africa. Technical Communication No. 193, Department of Agriculture, Republic of South Africa.

KINGSBURY, J. M. (1964). Poisonous plants of the United States and Canada. Prentice Hall Biological Science Series, Englewood Cliffs, NJ, USA.

LAU, H. D. & SINGH, N. P. (1985). *Pesquisa Agropecuaria Brasiliera*, Brasilia, 20, 873–875

MUCHIRI, D. J., BRIDGES, C. H., VECKERT, D. N. & BAILEY, E. M. (1980). *Journal of the American Veterinary Medical Association*, 177, 353–354.
MURDIATI, T. B. & LOWRY, J. B. (1983). Toxicity of *Brachiaria decumbens*. In Research Report Project for Animal Research and Development, Balai Penelitian Ternak, Bogor, Indonesia. pp 56–57.
OTHMAN, A. M., YUSUFF, M. K. & ABDUL RAHMAN, S. O. (1983). *Malaysian Veterinary Journal*, 7, 254–263.
OTHMAN, A. M., HARON, S. YUSUFF, M. K. & SAAD, M. A. (1983). *Malaysian Veterinary Journal*, 7, Abstract 271.
PIERRE, F. (1984). *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, 37, 277–285.
TEMPERINI, J. A. & BARROS, M. A. (1977). *Biologico*, 43, 103–110.
TOKARNIA, C. B., DOBEREINER, J. & DA SILVA, M. F. (1979). Plantas toxicas da Amazonia a bovinos e autros herbivores. Instituto Nacional de Pesquisas da Amazonia.

Research Note

GROWTH PATTERN OF DESI (INDIGENOUS) AND CROSSBRED CHICKS UNDER RURAL SCAVENGING CONDITIONS

M. H. SAZZAD, S. M. H. MAMOTAZUL and M. U. ASADUZZAMAN

Livestock Research Institute, Savar, Dhaka, Bangladesh

In Bangladesh poultry are generally raised in rural areas as scavengers and as a consequence they are not very productive. In a previous study it was found that the average body weight of adult desi hens is 890 g ± 134 and annual egg production per hen is 45 (Sazzad, 1986).

Various crossbreeding programmes have been undertaken to improve the performances of poultry under rural conditions. The current study was undertaken to ascertain the effectiveness of one of these programmes. Sixty small households were selected in two villages near Savar, Dhaka, an area where local desi birds have been crossed with White Leghorns, Rhode Island Reds and Fayoumi. Male and female birds were weighed each week. The average weight of the desi males at eight weeks was 205 g ± 46 and that of the females 189 g ± 78. The crossbreds achieved similar weights and the differences in growth rate between desi and crossbreds were not significant.

According to Mukherjee (1987) non-genetic influences such as poor nutrition have a much higher effect on production parameters of poultry than genetic influences. From the results of this investigation it is confirmed that crossbred chicks under scavenging conditions do not have higher growth rates and productivity than desi poultry.

Accepted for publication February 1989

REFERENCES

MUKHERJEE, T. K. (1987). Problems and prospects of genetic improvement of poultry for rural areas in Asia. Paper presented at the FAO expert consultants meeting for rural poultry development in Asia at Dhaka, Bangladesh, 1987.
SAZZAD, M. H. (1986). Reproductive performance of the desi hen under scavenging and intensive system of rearing. Proceedings of First Annual Livestock Research Workshop. Livestock Research Institute, Savar, Bangladesh, pp 60–62.

BOOK REVIEW

Newcastle Disease. Ed. D. J. Alexander. Developments in Veterinary Virology (series). Martinus Nijhoff Publishing, The Netherlands. 1988. 378 pp. £71.25. US$120. ISBN 0-89838-392-7.

The preface indicates that the purpose of the book is to give a comprehensive account of the virus, the disease and its control. It is aimed at those interested in research and in the application of diagnostic and control methods.

For this objective, 22 international experts have contributed to this volume of 19 chapters each of which is followed by numerous references. Initially there is a succinct account of the history of the disease and then a chapter on taxonomy with the classification of the virus within the genus paramyxovirus. Four chapters follow describing the molecular basis of structure, replication, gene cloning and nucleotide sequencing, and infectivity and pathogenicity. The heterogeneity within strains of the virus and its significance for virus survival is considered and then the value of monoclonal antibodies in research, diagnosis and epizootiology. The next three chapters deal with disease: the first describes the pathogenesis, signs and gross and histological lesions in the fowl, turkey, duck, goose and pigeon; the second considers panzootic ND in pigeons and the third ND in free-living pet birds with a comprehensive table of the species of birds involved. A chapter on geographical distribution of the virus is followed by one on methods of spread and an account of the disease in tropical and developing countries. The final four chapters consider control including policies, vaccination and vaccines and their quality control. A detailed index follows.

This book achieves its purpose and the subject matter is well presented throughout. However there are several minor criticisms, for instance, there is excessive repetition particularly in some of the earlier chapters, additional cross-referencing would have been beneficial, the quality of some of the black and white photographs is not in keeping with the high standard of the text and there is no mention of food pellet vaccination in the control of disease. Nevertheless this book is of inestimable value to all involved with this subject and is a fitting token of remembrance to the late Professor R. P. Hanson to whom it is dedicated.

F. T. W. Jordan

APPENDIX III. ENDOPHYTES IN CASSAVA

Preliminary results

The existence of detrimental endophytes affecting cassava was demonstrated in 1990 (Annual Report 1991), and a system was developed to evaluate the effect of endophytes on cassava plantlets under control conditions in 1992 (Annual Report 1992). By following this system to test around 213 fungal strains isolated from the rhizosphere of different cassava clones planted in different ecological areas, three strains belonging to different fungal species had a beneficial effect on cassava plantlets inoculated by three inoculation methods. Their effect on root weight increase in three clones (CG 1141-1; M col 1468 and M col 1505) was significant (0.05 Tukey' test) for the three inoculation methods used when compared with controls (Fig. 1.1) as well as when three cassava clones were inoculated with each of three strains used (Fig. 1.2). However, strain E1 induced the highest root-yield both for all inoculation methods used (Fig. 1.1) and in all inoculated clones (Fig.. 1.2). The existence of beneficial endophytes parasitizing cassava is important as this opens the possibility of increasing biomass production by direct application of these endophytes to plantations or by inducing indirect plant protection against detrimental parasites after applying transformed beneficial endophyte strains.

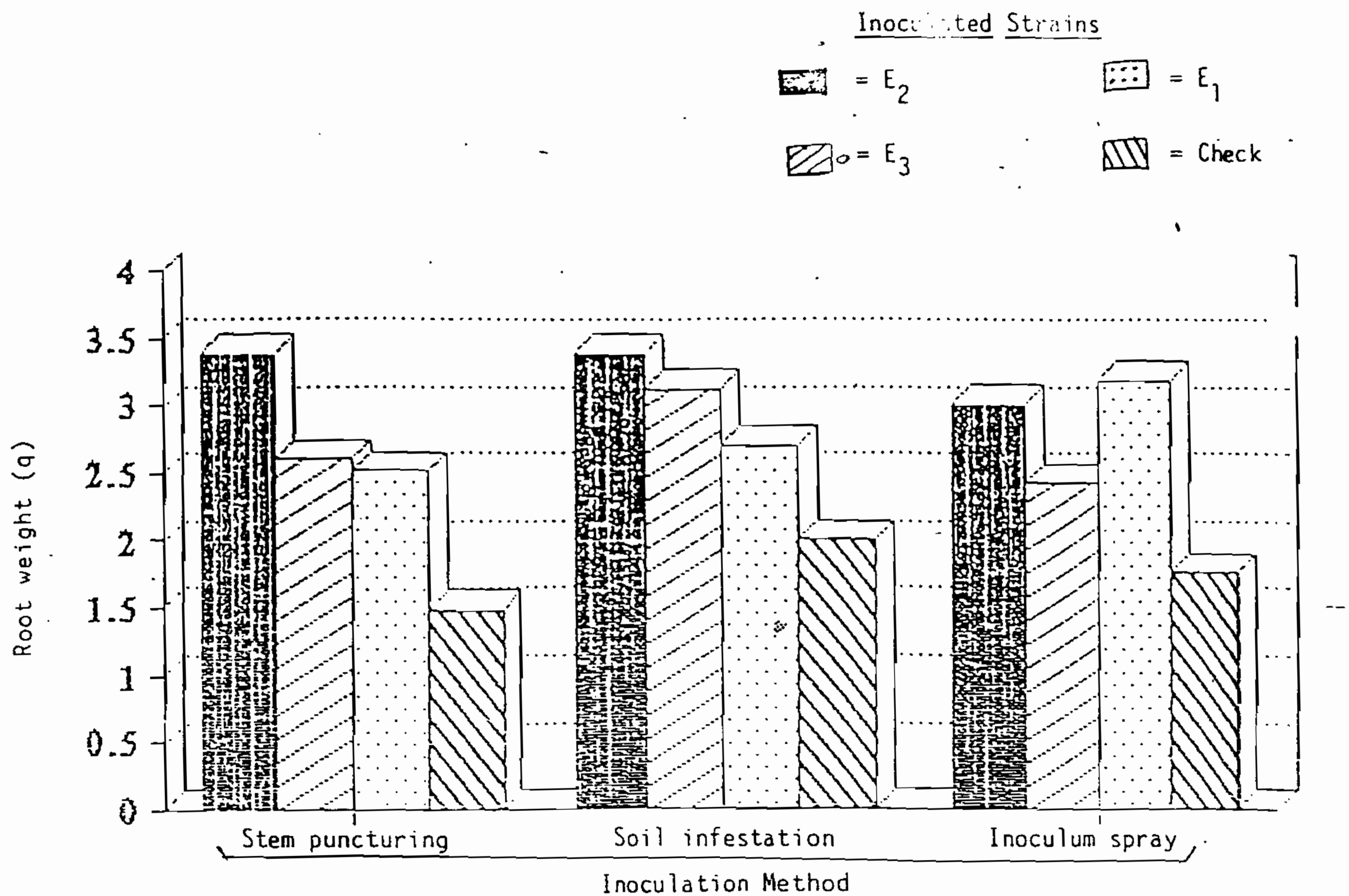


Figure 1.1. Effect of three endophyte species on root weight of one-mo-old plants of three different clones (CG 1141-1, M Col 1418 and M Col 1505) inoculated by stem puncturing, soil infestation and inoculum spray with a mycelia suspension equivalent to 2 petri dishes/500 ml of distilled water.

Source: Cassava Pathology/CIAT; Cassava Program Annual Report (22 Nov. 1993, Draft)

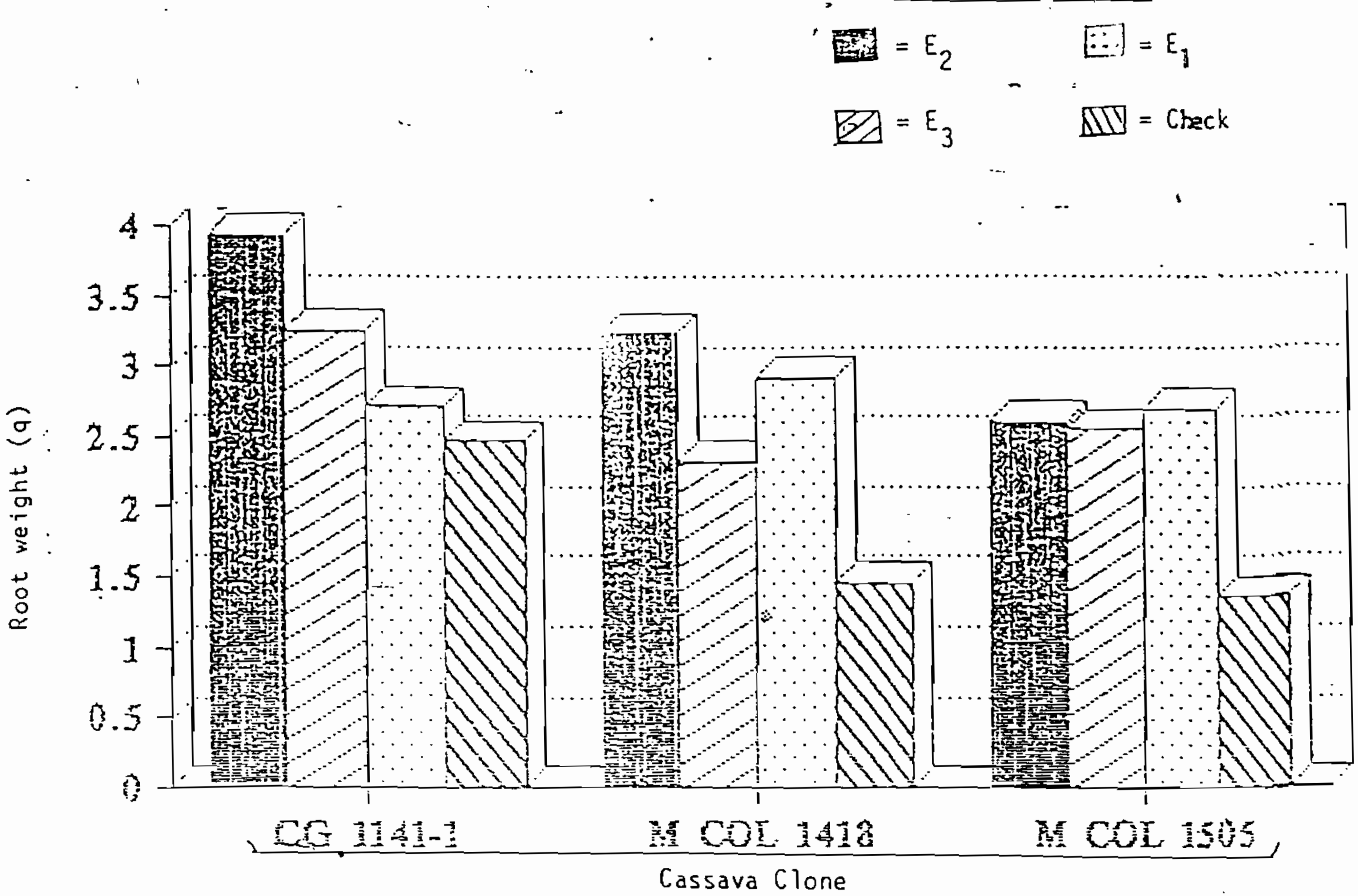


Figure 1.2. Effect of three endophyte species on root weight of plantlets of three cassava clones inoculated by three inoculation methods (stem puncturing, soil infestation and inoculum spray).

Source: Cassava Pathology/CIAT; Cassava Program Annual Report (22 Nov. 1993, Draft)