IOBC / WPRS

Working Groups
"Integrated Control of Cereal Crops"
and
"Integrated Control in Oilseed Crops"

Les Groupes de Travail
"Lutte Intégrée en Céréales"
et
"Lutte Intégrée en Cultures d'Oléagineux"

The 2nd International Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf

Paderborn (Germany)
22 - 24 November 1995

Edited by

Karsten Krohn, Volker H. Paul

IOBC / wprs Bulletin
Bulletin OILB / srop Vol. 19(7)1996

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Régionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat:
INRA Station de Zoologie
Domaine Saint-Paul
Site Agroparc
84914 AVIGNON Cedex 9
France

ISBN 92-9067-081-9

Preface

The first Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf, held at Paderborn (Germany) in 1993, demonstrated the great demand for information in research and practical application. Therefore, the organizers decided, encouraged by many participants, to establish the Conference as a regular meeting.

The 2nd Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf was held in Paderborn from November 22-25, 1995. We succeeded in establishing an international scientific network involving two working groups of the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) (WG: Oilseed crops, convener: V.H. Paul; WG: Cereal Crops, convener: H.M. Poehling), and the research area on secondary metabolites (coordinator: K Krohn, Faculty of Chemistry, Paderborn). The main subjects of the Conference were: "Harmful and beneficial microorganisms", "Secondary metabolites","Product quality", "Genetic engineering and biotechnology", "Resistance breeding" and "Plant protection and environment".

The continuation of this conference will ensure a widely faceted scientific and practice-related exchange of the latest research results not only within Europe but also worldwide.

At the end of the 2nd Conference a large majority of the participants voted for Paderborn as the location for the next meeting. This presumably will be in spring 1998.

We would like to thank Iris Föller, Mariola Zukowski, Karsten Beckmann, Carsten Biele, Peter Dapprich, Karl-Heiz Drogies, Michael Henneken, Markus John and Johannes Reinholz for their help in the organization and Oliver Kamp for his assistance in the computer generated compilation and uniform formation of the original manuscripts.

Karsten Krohn and Volker H. Paul

Contents

Opening Adress

Opening lecture

Harmful and Beneficial Microorganisms

Secondary Metabolites

Product Quality

Gene- and Biotechnology

Resistance Breeding

Crop Protection and Environment

Opening Lecture

Address
of the Parliamentary State Secretary
of the Federal Ministry of Food, Agriculture and Forestry

Wolfgang Gröbl

on the occasion of the 2nd International Conference on Harmful and Beneficial Microorganisms in Pastures, Turf and Grassland
21-23 November 1995 (Paderborn)

When I gave a welcoming address to the participants attending the first international conference on harmful and beneficial microorganisms in pastures, turf and grassland in 1993, the content and the significance of this conference led me to realise that such a conference would meet with a wide response in the fields of research and practical application. I am now pleased to see that my impression was correct. This conference is now taking place in Paderborn for the second time, with experts from 12 countries coming together for three days to discuss topics relating to agricultural and environmental aspects in the grass sector.

Permanent Grassland accounts for 40% of the entire agricultural area of the European Union (31% is the respective figure for the Federal Republic of Germany) and thus constitutes an important economical as well as ecological factor. It can be expected that the importance of this sector will increase even further within the course of the implementation of the common european agricultural policy.

According to the programme of this years conference, important fields such as "Harmful and beneficial microorganisms", "Secondary metabolites", "Product quality", "Genetic engineering and biotechnology", "Resistance breeding" and "Plant protection and the environment" are topics for discussion. Special importance will have to be dedicated to the necessary objective, scientific treatment of topics such as genetic engineering,

biotechnology, plant protection and the environment which are often the subject of public controversy.

The subject of "Endophytic fungi in grasses" is also of topical interest. This theme, the subject of intense discussions particularly overseas, is still relatively unknown here in Germany. It could though become significant regarding the improvement of natural resistance in grasses to certain biotic and abiotic stress factors. The potential toxicity of some ingredients is a problem with endophytes which must not be underestimated, especially under the changing climatic conditions. On endophytes, and especially their influence on the quality of fodder, as well as their presence in general, the information available in Germany is also inadequate.

The fact that this conference was included into the EUCARPIA programme (European Association for Research on Plant Breeding) as an official workshop indicates the international significance of this topic and also the acceptance of this conference, which is to be welcomed. The continuation of this conference every three years will ensure a widely faceted scientific and practice-related exchange of the latest research results not only within Europe, but also worldwide. I am convinced that this will enable Europe and particularly Germany to contribute valuable research results in the area of grassland and grass cultivation and to keep up with international developments.

The Changing Grassland Scene - A Challenge For The Scientist

M.D.Hayward

Institute of Grassland and Environmental Research, Aberystwyth, Wales, UK.

ABSTRACT

The changing grassland scene in Europe as a result of altered political and sociological pressures poses many challenges to the grassland scientist. Some of the areas of concern include the requirement to develop sustainable farming systems in order to meet the demands for environmental acceptability and the desire of the consumer for greater biodiversity and for differing uses of grassland.

Keywords: grassland, sustainability, nitrates, breeding, clover, biodiversity.

Introduction

What is the future for grassland farming in Europe? This is a question currently being asked by all of those involved in farming irrespective of whether it be the intensive cereal producers, the olive oil grower, the livestock farmer or any of the diverse sectors that are to be encountered across the European farming scene. In many respects each of these farming activities are under considerable pressure of one form or another but it is the grassland scene which is perhaps subject to the greatest change as we go into the next century. Many factors are operating on the direction of agriculture, and as far as we in Europe are concerned, the strongest without doubt, is the Common Agricultural Policy (CAP) of the European Union with its concern with over-production, set-a-side, quotas and environmental issues.

It is impossible to predict what future direction these 'political pressures' will take other than to say that they can affect the pattern of agriculture to a very great degree both in the short and long term. Irrespective of these CAP based influences there are, in addition,

increasing pressures, particularly from the environmental lobby, to develop farming systems which are more 'sustainable' in the long term. This applies particularly to the grassland sector which is now recognised as being of overriding importance in the 'environmental' scene.

In addition to these political pressures the whole question of consumption of meat and milk in the future will strongly influence the requirement for livestock products. For sociological and moral reasons the consumption of meat has declined over recent years with vegetarianism on the increase, particularly in the younger generations. It has been predicted by the UK Ministry of Agriculture that consumption of beef and veal will decrease by about 15% between now and the early years of next century. The recent problems in the UK over the export of livestock clearly illustrates this growing trend. Such factors will again have an important impact on the grassland scene.

Sustainability in the grassland scene

Before considering some possible changes in the grassland scene in Europe and the role which the grassland scientist may have in shaping it, sustainability in the context of grassland farming should be addressed. In general it may be said that it embraces a number of objectives: resource efficiency, profitability, productivity, environmental soundness and social viability (Francis & Callaway, 1993). In the grassland scene it aims to improve the overall quality of grassland farming, its integration into a more environmentally acceptable rural economy and to meet the needs of changing consumer demand.

What are the particular problems and the likely requirements to solve them? These topics will be considered in a somewhat general context and some of them addressed from the point of view of grassland science and as to how it may contribute to the development of these sustainable systems.

Requirements for sustainable grassland farming

The primary requirement of any grassland system is to provide feed for ruminant farm animals. The output from grass is generally measured in terms of livestock products, milk, meat, hides and wool. In the future however we may be looking at the output from grassland not just in terms of these primary products but also in the context of alternative

uses such as for biomass for industrial processing or for fuel and, over and above this, in terms of its amenity value as a landscape.

Agricultural Grassland.

As already indicated the future patterns of production of livestock production from grassland will be strongly influenced by political pressures coming from the Common Agricultural Policy of the European Union. These pressures will undoubtedly increase as enlargement of the Community to include the countries of eastern Europe occurs. These countries have considerable potential for raising the productivity of all sectors of their agriculture. As an example of the legislative changes which are taking place in Europe and are beginning to have a major influence on agricultural practises and as such pose challenges to the grassland scientist the '1991 Nitrates Directive of the Council Of Ministers of the EC may be considered as a prime example (Directives 75/440/EEC,79/869/EEC & 80/778/EEC).

This directive aims to reduce water pollution caused by nitrates from agricultural sources. The losses from grassland can be quite considerable where heavy applications of artificial nitrogen fertilizers are applied. Up to 66kg/ha/annum of nitrogen may be lost due to leaching, denitrification and volatilisation (Wilkins, 1994). The directive has recognised that for certain areas of land, particularly those which form the catchment areas of public water supply systems, that the nitrate content of the ground water should be below 50mg/l. For these nitrate sensitive areas the Act 'allows Ministers to prevent or control the entry of nitrate into controlled water as a result of anything done in connection with the use of land for any agricultural purposes'. So far it appears that upto 20% of the UK agricultural land area is at risk. The mandatory measures include rules relating to the periods when land application of certain types of fertiliser both chemical and natural, including sewage sludge, is prohibited. For each farm the amount of livestock manure applied to land each year will not exceed a specified amount per hectare. In other words the effect will be that the livestock carrying capacity of farms will be determined according to their ability to dispose of effluent without adversely affecting nitrate concentration of water courses. What are the effects of such legislation going to be on the overall grassland scene and again what challenges do they pose.

There are several options available to the livestock farmer to ameliorate this problem.

Firstly he may reduce his stocking rate in other words a more extensive system of farming may develop. A change will take place on those farms which have traditionally been intensive users of applied nitrogen such as the dairy farms of Britain and the Netherlands. Some farms may go out of milk production altogether (Here the imposition of milk quotas will play an important role). The land will be used for alternative livestock such as beef or sheep or may go out of livestock production all together and be used for completely different enterprises such as for biomass or forestry. It seems likely that we shall see a polarization of production patterns: on the one hand farms with low inputs, and with consequential low outputs per hectare and on the other highly intensive dairy farms with high inputs and high outputs per hectare provided they do not have an effluent disposal problem. These farms will undoubtedly rely on an input of grass but arable crops such as forage maize will play an important role in maintaining production.

From the grassland point of view what can the grassland scientist contribute in order to maintain productivity and sustainability? As an example one can consider the potential of breeding for improved nitrogen use efficiency. Before considering this it should be emphasised that temporary grassland in Europe will continue to be dominated by the ryegrasses *Lolium perenne* and *Lolium multiflorum.* Tall fescue (*Festuca arundinacea)* and Orchard Grass (*Dactylis glomerata)* will be used to some extent in southern and eastern Europe whilst Meadow Fescue (*Festuca pratensis)* and Timothy (*Phleum pratense)* will be used in the northern parts. Some novel intergeneric hybrids and genetically modified forms may however have an increasingly important role to play. These in their own right will create problems and challenges before they can be accepted.

Breeding for improved nitrogen recovery in grasses.

At IGER we have been looking at the potential for producing cultivars of ryegrass which are more efficient at taking up any applied nitrogenous fertilizers. In other words can pollution be minimized by using cultivars which recover more of the applied nitrogen hence there is less to contaminate the ground-water.

In the Grass Breeding Group at IGER Dr. P.W. Wilkins and Mr A. Lovatt undertook an assessment of a range of cultivars of *Lolium perenne* to ascertain whether there was genetic variation in the recovery of applied nitrogen as expressed through total herbage yield as opposed to nitrogen content *per se*. They were able to identify some novel germplasm which did indeed have this attribute. Appropriate genotypes have been selected to form the parents of a synthetic cultivar (Aber Elan) which is now to be marketed with this desirable characteristic.

Depending on the farming practise it may be used in high production systems enabling output to be maintained but with lower inputs or with inputs at even higher levels to increase productivity. This latter situation, making better use of 'natural soil nitrogen', will become of increasing relevance as we extend the use of grass/clover based pastures.

Here there is a challenge facing both the breeder and agronomist There is a need to develop farming systems which will accommodate the new cultivars of grass and clover in order to increase farming efficiency and hence sustainability.

The contribution that clover based systems can make to overall sustainability, particularly in the area of reduced pollution and lower inputs is one which needs to be addressed and some problems solved before clover is more widely accepted.

Professor Roger Wilkins at IGER has shown that clover based pastures are more environmentally acceptable both from the direct polluting aspects and also in terms of the support energy that goes into the whole farming system (Table 1.).

Table 1. Environmental Impact of Nitrogen Based versus Clover Based Pastures

Normal	Clover Based (no N)
Losses of N (kg/ha/ann)	
Total 66	42
Greenhouse Gases (CO_2)	
577	524
Support Energy (GJ)	
13	3

(After Wilkins, 1994)

The reluctance of farmers to adopt clover has, in the past, been mainly due to the problems of unreliability of clover particularly in the areas of establishment and the possible problems of production in spring and the risk of bloat in the high yielding dairy cow.

The Breeding of more effective clovers/Rhizobium.

An area of particular challenge in enhancing the prospects for clover is to gain a better understanding of the interaction of grass, clover and the symbiotic *Rhizobium*. Clover breeders and soil microbiologists have started to tackle some of these problems such as the difficulty of establishing a good percentage in the pasture, its limited growth in early spring and the lack of persistence. Attendant with this has been the recognition that it is important to identify the right cultivar of companion ryegrass to associate with the clover in order to ensure the longevity of the clover in the pasture (Rhodes & Webb, 1993) . There is however still a requirement to gain an understanding of the genetically determined physiological mechanisms that contribute to grass/clover compatibility. Similarly it is well known that both the genotype of the clover plant and its companion *Rhizobium*, can influence the efficiency of nitrogen fixation. Both of these are amenable to manipulation by the breeder and biotechnologist. As such we can look forward to the selection of complementary combinations of this symbiosis which can raise the level of productivity of the pasture, particularly the grass component.

The problem of bloat is one that the biotechnologists are tackling. The absence of tannin production in *Trifolium repens* is the main reason for the induction of bloat. Work is in progress to modify the biosynthetic pathway in order to overcome this by the use of antisense gene technology and transformation. (Morris & Robbins, 1995). These transformation techniques also offer the prospects of producing clovers which are more resistant to insect pest and diseases. In this respect the genetic manipulation of the symbiotic *Rhizobium* offers a unique means of introducing some resistance into the clover host plant such as for the *Sitona* weevil (Skøt, *et al.* 1994).

Each of these developments individually offer the prospect of answering some of the current problems being considered by the grassland scientist but they still need to be combined together into a holistic approach in order to meet the increasing forces that are acting upon the grassland scene at large.

Alternative roles for Grassland

In prospect, grassland farming, where centred around livestock production, will continue to exploit the somewhat traditional approach but as mentioned earlier there will probably be a polarization towards the intensive and the extensive. This latter will undoubtedly become of increasing relevance in terms of the maintenance of 'landscape' and nature conservation.

In Europe we are already seeing areas of the countryside being identified as 'Environmentally Sensitive' or of 'Special Scientific Interest'. Over the past 40 or so years there has been a series of developments such as the establishment of National Parks, the designation of Environmentally Sensitive Areas and so on which are all aimed at the maintenance of the rural landscape. In order to maintain a landscape we need to consider the many diverse factors operating upon it and at the same time to remember that it is a working environment for the farmer. First and foremost we cannot divorce the landscape as we see it today from what has happened in agriculture over the past century or more. The average urban dweller thinks of the countryside as a green fields with hedgerows, open hills and moorlands with sheep and lambs grazing, as meadows filled with wild flowers not the monotony of a single cultivar field of ryegrass regularly cut for silage. The role of grassland in maintaining biodiversity is now more widely recognised not just at the plant level but also in its influence upon the wide range of insects and birds which it can support.

It is this type of landscape that people want to see maintained. The question is of course are they prepared to pay for the privilege of seeing such scenes? It is also a challenge to the grassland agronomist to develop sustainable farming systems which will meet these requirements. National governments and the European Union now realize that in order to maintain this rural idyll that the working farmer must be paid in order to keep him in the countryside. Several schemes are in operation to make payments to farmers to maintain the landscape. The ESAs are one example where farmers are paid not to apply fertilizers.

Alternative uses of grassland.

Grass as we understand it today has very few attributes which make it attractive as a possible industrial crop. However there is increasing interest in developing industries to use grass either as biomass for fuel or for processing for paper.

A lot of interest is being expressed in northern Europe to use *Phalaris* as a possible source of fibre for the paper industry. Here there is a challenge for the biotechnologist to genetically modifying the lignin/cellulose pathways to enhance utility even of such species as ryegrass for both of these purposes.

Impact of Biotechnology.

The prospects of genetic modification of all components of the grassland biome is perhaps the major challenge facing the grassland scientist. New opportunities develop almost daily for the production of some novel organism and with it the potential for changing the grassland scene. The possibilities are considerable and include a wide diversity of plant and animal processes. The production of such transgenics as herbicide tolerant *Agrostis sp.* and *Festuca sp.* could be beneficial for the management of the hill land environment to reduce the spread of bracken (*Pteridium*) if grazing pressures are reduced.The potential for modification of *Acremonium sp* for pharmaceutical production is creating a lot of interest. In this context the major challenge as I see it is going to be assessing the environmental impact of the introduction of such transgenics into grassland agriculture.

Conclusions

Some of the facets of the changing grassland scene have been outlined. The areas mentioned are by no means exhaustive in terms of the challenges that they pose. Whatever challenges are considered , they will require close cooperation between all grassland scientists in order to provide the answers to the increasing demands being put upon the grassland scene. It must be emphasised that these challenges and their solution will be subjected to even greater scrutiny as we go into the next century, as such, we must be prepared to satisfy our customers - the public.

References

Francis C.M.and M.Brett Callaway, 1993. Crop improvement for Future Farming Systems. In *'Crop Improvement for Sustainable Agriculture'*. Ed . M.Brett Callaway and C.A.Francis. Univ Nebraska Press. Lincoln. 1-18.

Morris P. and Robbins M, 1995. Manipulating condensed tannins in forage legumes. In *'Biotechnology and the improvement of legumes'* . Ed. B.D. McKersie, and D.C.W. Brown, CAB International - in press.

Skøt L., Timms E. and Mytton, L.R. 1994. The effect of toxin producing *Rhizobium* strains on larvae of *Sitona flavescens* feeding on legume roots and nodules. *Plant and Soil.* 163, 141-150.

Rhodes I. and Webb K.J. 1993. Improvement of White Clover. *Outlook on Agriculture*. 22, 189-194.

Wilkins R.J. 1994. The Future of Grassland Farming - A Scientists View. *Journal Agric. Soc. Univ. Wales.* 74. 53-64.

Harmful and Beneficial Microorganisms

A review of research on endophytic fungi worldwide, and its relevance to European grassland, pastures and turf

G.C. LEWIS

Institute of Grassland and Environmental Research, North Wyke Research Station, Okehampton, Devon EX20 2SB, UK

ABSTRACT

Research on seed-borne infection of temperate grasses with endophytic fungi is reviewed. Emphasis is placed on the extensive research on *Festuca arundinacea* infected with *Acremonium coenophialum* and *Lolium perenne* infected with *A. lolii*, in the USA and New Zealand respectively. The great importance of these associations to the agriculture and turf industries is discussed, particularly in relation to the production of alkaloid compounds in infected plants. The activities of these alkaloids are outlined, including deterrence of feeding by insect pests, and toxicity to grazing mammals. Other attributes of endophyte-infected plants are discussed, including improved tolerance of stress.

It is proposed that the apparent absence of marked effects of endophyte infection in European grassland is due largely to the low levels of infection in seed of European-bred cultivars of *L. perenne*. Data are presented to show that endophyte infection is common and widespread in long-established grassland in many European countries. The need to evaluate the potential for pest control in Europe is discussed, in the knowledge that the species of insect deterred from feeding on endophyte-infected *L. perenne* in New Zealand do not occur in Europe. Evidence is presented to support the hypothesis that *A. lolii*-infected *L. perenne* increases tolerance to stress from biotic and abiotic factors.

Keywords: Endophytic fungi, *Acremonium* spp., *Epichloë* spp., forage and turf grasses, *Lolium* spp., *Festuca* spp., alkaloids.

ENDOPHYTIC FUNGI WORLDWIDE

The presence of endophytic fungi in grasses has been reported from virtually all continents of the world, and many different endophyte/grass associations exist (White, 1987). The associations most commonly found in temperate grassland are shown in Table 1. *Epichloë* species reproduce sexually and cause the disease in grasses known as 'choke', in which a stroma is produced on a fertile tiller, preventing the inflorescence from emerging. Until recently, there was one species, *E. typhina*, with a wide host range, but it is now known that different species of *Epichloë* infect different grass species (Leutchmann, 1994; White, 1993). *Acremonium* species reproduce asexually, infection is symptomless and seed-borne, and the association between fungus and host is regarded as mutualistic, i.e. both benefit from the association (Hill, 1994).

Table 1. Main species of endophytic fungi and their host grasses found in temperate grassland.

Endophyte species	Grass host species
Epichloë typhina	*Dactylis glomerata*
E. clarkii	*Holcus lanatus*
E. baconii	*Agrostis capillaris*
E. festucae	*Festuca* spp
Acremonium lolii	*Lolium perenne*
A. coenophialum	*Festuca arundinacea*
A. uncinatum	*F. pratensis*
A. typhinum	*Festuca* spp

Research on endophyte/grass associations in temperate grassland has been concentrated on *Acremonium coenophialum*/*Festuca arundinacea* and *A. lolii*/*Lolium perenne*, in the USA and New Zealand, respectively. The reason for this situation is that these particular associations are of great significance to the agriculture and turf industries, mainly because of the alkaloid compounds that are produced in infected plants (Porter, 1994). There are four main groups of alkaloids (Table 2), and some alkaloids have beneficial effects whereas others have detrimental effects. Ergovaline present in infected

plants of *F. arundinacea* is the chief suspect in cases of toxicity in grazing animals that cause losses of $600 million per year in animal production in the USA (Hoveland, 1993). Lolitrem B in infected plants of *L. perenne* is the cause of 'ryegrass staggers', in which animals lose coordination of movement. Staggers is a major problem in New Zealand and Australia (van Heeswijck & McDonald, 1992). These significant adverse effects of alkaloids are counterbalanced by the beneficial effects of other alkaloids, notably lolines in *F. arundinacea* and peramine in *L. perenne*, which deter a variety of insect pests from feeding on the plants (Clement *et al*, 1994). However, the role of the various alkaloids in endophyte-infected grasses is unclear, and some alkaloids may be involved in both insect deterrence and toxicity to grazing animals.

Table 2. The four main groups of alkaloid compounds found in endophyte-infected grasses.

Ergopeptines	e.g. ergovaline, ergotamine
Indole diterpines	e.g. Lolitrems A,B,C,D
Pyrrolopyrazine	e.g. peramine
Pyrrolizidines	e.g. N-formyl loline, N-acetyl loline

Plants of *F. arundinacea* infected with *A. coenophialum* are more tolerant of drought stress and are more competitive than uninfected plants (West, 1994). The mechanisms involved are little understood. The overall benefits of endophyte infection in *F. arundinacea* and *L. perenne* are such that a high proportion of the seed used for agriculture and turf in the USA, New Zealand and some other countries contains endophyte infection. Thus the prime consideration is endophyte-enhanced grass persistence under stress; the persistence of endophyte-free grass is generally regarded as unacceptable. Strategies to reduce toxic effects on grazing animals are being developed, including animal and pasture management (Stuedemann & Thompson, 1993), treatment of animals (Thompson & Garner, 1994), breeding of resistant animals (Morris *et al*, 1995), and production of grass cultivars infected with strains of endophyte that do not induce animal toxins (Rolston, 1993).

One of the most important findings in recent years is that there can be a great variation in effects between individual endophyte-infected plants within a grass population (Hill, 1994). This variation is expressed in terms of, for example, fungal morphology in culture, alkaloid type and concentration, and effects on stress tolerance. Thus the effect of biotic and abiotic stress on a given grass population is likely to vary between the individual members of the population.

ENDOPHYTIC FUNGI IN EUROPE

All of the endophyte/grass associations listed in Table 1 are found in Europe. Indeed, European settlers are generally regarded to be responsible for inadvertantly introducing endophyte-infected seed of *L. perenne* to New Zealand and of *F. arundinacea* to the USA. In Europe, *L. perenne* is the most important grass species and many cultivars have been bred for use as forage and turf. From the limited information available, it appears that seed of these European-bred cultivars rarely contains significant levels of infection (Table 3). However, infection is common and widespread in wild populations of *L. perenne* in many European countries (Table 4).

Table 3. Proportion of seeds of European-bred cultivars of *L. perenne* infected with *A. lolii*

No. of cultivars examined	No. with infection	Reference
68	8	Dapprich *et al* 1994
38	1	Lewis & Clements, 1986
16	4	Latch *et al* 1987

The status of *A. lolii*-infected *L. perenne* in Europe differs greatly from that in New Zealand. In New Zealand, the vast majority of pasture is dominated by *A. lolii*-infected *L. perenne*, because infected seed has been used to establish these pastures. In Europe, however, the cultivars used in the last 15-20 years appear to have been largely endophyte-free (Table 3). Probably, loss of infection is due to low levels of infection in the breeding material, accompanied by loss of fungal viability in seed during storage (Rolston *et al*, 1986). Wild populations of *L. perenne* in Europe can contain a high

proportion of infected plants (Table 4) but these populations often are only a minor component of a sward. Therefore the effect of alkaloids produced by infected plants can be diluted by the presence of other, uninfected plant species.

Table 4. Percentage of wild populations of *Lolium* spp. infected with *Acremonium* endophytes.

Country	% of populations with infection	Reference*
Austria	0	1
Belgium	28	1 & 2
Bulgaria	78	1
Czech Rep	67	1
England	70	1 & 2
France	72	1
Germany	87	3
Italy	46	1
Norway	0	1
Portugal	100	1
Romania	93	1 & 2
Slovakia	20	1
Spain	75	1
Switzerland	12	1 & 2
Turkey	66	1
Wales	78	1 & 2

* 1. G.C. Lewis & C. Ravel, unpublished;
2. Latch *et al*, 1987; 3. Oldenburg, 1994

In New Zealand, the high levels of *A. lolii*-infected plants of *L. perenne* in pasture represent a high risk to grazing animals. The occurrence of 'ryegrass staggers' is linked strongly with periods of hot, dry weather in late summer, when grass growth ceases and the concentration of Lolitrem B increases (Ball *et al*, 1995). When grass growth resumes after rainfall, animals grazing the new flush of growth are especially prone to staggers. Such climatic conditions occur annually in many areas in New Zealand, but are relatively

infrequent in most areas of Europe. In 1995, however, a long summer drought in England caused a marked increase in reported cases of staggers (Pritchard & Lewis, 1995).

The pests that are deterred from feeding on *A. lolii*-infected plants of *L. perenne* in New Zealand are not found in Europe, and no deterrence has been reported for insects indigenous to Europe. The major pests of *L. perenne* in Europe probably are frit fly (*Oscinella frit*), nematodes (especially *Meloidogyne naasi* and *Pratylenchus penetrans*), leatherjackets (*Tipula* spp larvae) and aphids (especially *Rhopalosiphon padi*). Frit fly appears to be unaffected by *A. lolii*-infected *L. perenne* (Lewis & Clements, 1986), but research is needed to investigate the potential of endophyte infection for biological control of other crops.

There is some evidence that infection by *A. lolii* increases tolerance of *L. perenne* plants to stress caused by biotic and abiotic factors. In plants of one genotype of *L. perenne*, the presence of *A. lolii* significantly increased herbage yield when the plants were also infected with a virus, and the increase was greater with plants given severe defoliation (Lewis, 1996). Also, an association between infection with *A. lolii* and increased tolerance of drought stress in *L. perenne* was apparent in wild populations in France (Grand-Ravel *et al*, 1996).

Infection of *F. pratensis* by *A. uncinatum* induces the production of lolines (Bush & Schmidt, 1994) and these alkaloids have been associated with deterrence of feeding by root and leaf aphids and other insect pests (Schmidt, 1994). Also, the presence of *A. uncinatum* in seeds increased susceptibility to pre-emergence death of seedlings caused by some fungal pathogens, but decreased susceptibility to post-emergence infection by other fungal pathogens (Schmidt, 1994). Observations of various grasses in field plots in Switzerland have shown that the presence of endophyte infection has caused marked reductions in infection by rusts (*Puccinia* spp.) and other foliar fungal pathogens (D. Schmidt, personal communication).

CONCLUSIONS

The effects of endophyte-infection in grasses are well documented in the USA and New Zealand, but the impact of infection in European grassland is largely unknown. The

large areas of long-established grassland (some of which is centuries old) in Europe provide a rich source of endophyte-infected grasses and there is a need to explore this resource and assess the effects, both beneficial and detrimental. The exploitation of endophytes in agriculture and turf, and in other areas such as pharmacology (Porter, 1994), presents a major technological challenge, given the complexity of endophyte/grass associations and the wide range of chemicals produced. There is the potential for creating novel endophyte/grass associations, for genetically transforming the fungus, and for utilizing the fungus as a carrier of other genes. This work is already in progress in other countries (Siegel & Bush, 1994) and it is hoped that researchers in Europe will also take up the challenge.

REFERENCES

Ball, O.J-P.; Prestidge, R.A.; Sprosen, J.M. 1995: Interrelationships between *Acremonium lolii*, peramine and Lolitrem B in perennial ryegrass. *Applied and Environmental Biology*, **61**: 1527-1533.

Bush, L.; Schmidt, D. 1994: Alkaloid content of meadow fescue and tall fescue with their natural endophytes. *Proceedings of the Conference on Harmful and Beneficial Microorganisms in Pastures, Turf and Grassland, Paderborn, Germany, October 1993:* 259-265.

Clement, S.L.; Kaiser, W.J. ; Eichenseer, H. 1994: *Acremonium* endophytes in germplasms of major grasses and their utilization for insect resistance. In: *Biotechnology of Endophytic Fungi of Grasses.* (eds C.W. Bacon & J.F. White Jr), CRC Press: 185-199.

Dapprich, P.; Klose, A.; Paul, V.H. 1994; A survey on the occurrence of endophytic fungi in European seed lots of *Lolium perenne. Proceedings of the Conference on Harmful and Beneficial Microorganisms in Pastures, Turf and Grassland, Paderborn, Germany, November, 1993: 131-137.*

Grand-Ravel, C.; Astier, C.; Naafa, W.; Guillaumin, J-J. 1996: Relationships between climatic data and endophyte infection in France. *Proceedings of the Conference on Harmful and Beneficial Microorganisms in Pastures, Turf and Grassland, Paderborn, Germany, November, 1995.*

Hill, N.S. 1994: Ecological relationships of Balansiae-infected graminoids. In: *Biotechnology of Endophytic Fungi of Grasses.* (eds C.W. Bacon & J.F. White Jr), CRC Press: 59-71.

Hoveland, C.S. 1993: Importance and significance of the *Acremonium* endophytes to performance of animals and grass plant. *Agriculture, Ecosystems and Environment*, **44**: 3-12.

Latch, G.C.M.; Potter, L.R.; Tyler, B.F. 1987: Incidence of endophytes in seeds from collections of *Lolium* and *Festuca* species. *Annals of Applied Biology* **111**: 59-64.

Leutchmann, A.; Schardl, C.L.; Siegel, M.R. 1994: Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. *Mycologia*, **86**: 802-812.

Lewis, G.C. 1996: Effect of cutting height on perennial ryegrass with and without infection with endophyte and ryegrass mosaic virus. *Proceedings of the Conference on Harmful and Beneficial Microorganisms in Pastures, Turf and Grassland, Paderborn, Germany, November, 1995.*

Lewis, G.C.; Clements, R.O. 1986: A survey of ryegrass endophyte (*Acremonium lolii*) in the U.K. and its apparent ineffectuality on a seedling pest. *Journal of Agricultural Science* **107**: 633-638.

Morris, C.A.; Towers, N.R.; Wheeler, M.; Amyes, N.C. 1995: A note on the genetics of resistance or susceptibility to ryegrass staggers in sheep. *New Zealand Journal of Agricultural Research*, **38**: 367-371.

Oldenburg, E. 1994: Occurrence of fungal endophytes in *Lolium perenne. Proceedings of the Conference on Harmful and Beneficial Microorganisms in Pastures, Turf and Grassland, Paderborn, Germany, October 1993:* 99-104.

Porter, J.K. 1994: Chemical constituents of grass endophytes. In: *Biotechnology of Endophytic Fungi of Grasses.* (eds C.W. Bacon & J.F. White Jr), CRC Press: 103-123.

Pritchard, G.C.; Lewis, G.C. 1995: Ryegrass staggers. *The Veterinary Record* **28 October 1995**: 471-472.

Rolston, M.P. 1993: Use of endophyte in plant breeding and the commercial release of new endophyte-grass associations. *Proceedings of the Second International Symposium on Acremonium/Grass Interactions, New Zealand, February 1993*: 171-174.

Rolston, M.P.; Hare, M.D.; Moore, K.K.; Christensen, M.J. 1986: Viability of *Lolium* endophyte fungus in seed stored at different moisture contents and temperatures. *New Zealand Journal of Experimental Agriculture*, **14**: 297-300.

Schmidt, D 1994: Du nouveau sur les endophytes de la fétuque des prés. *Revue suisse Agriculture*: **26**: 57-63.

Siegel, M.R.; Bush, L.P. 1994: Importance of endophytes in forage grasses, a statement of problems and selection of endophytes. In: *Biotechnology of Endophytic Fungi of Grasses.* (eds C.W. Bacon & J.F. White Jr), CRC Press: 135-150)

Stuedemann, J.A.; Thompson, F.N. 1993: Management strategies and potential opportunities to reduce the effects of endophyte-infested tall fescue on animal performance. *Proceedings of the Second International Symposium on Acremonium/Grass Interactions, New Zealand, February 1993*: 103-114.

Thompson, F.N.; Garner, G.B. 1994: Vaccines and pharmacological agents to alleviate fescue toxicosis. In: *Biotechnology of Endophytic Fungi of Grasses.* (eds C.W. Bacon & J.F. White Jr), CRC Press: 125-131.

West, C.P. 1994: Physiology and drought tolerance of endophyte-infected grasses. In: *Biotechnology of Endophytic Fungi of Grasses.* (eds C.W. Bacon & J.F. White Jr), CRC Press: 87-99.

White, J.F. Jr. 1987: Widespread distribution of endophytes in the Poaceae. *Plant Disease*: **71**: 340-342.

White, J.F. Jr. 1993: *Epichloë* and *Acremonium* endophytes of grasses: systematic and experimental studies. *Proceedings of the Second International Symposium on Acremonium/Grass Interactions, New Zealand, February 1993*: 47-57.

van Heeswijck, R.; McDonald, G. 1992: *Acremonium* endophytes in perennial ryegrass and other pasture grasses in Australia and New Zealand. *Australian Journal of Agricultural Research*, **43**: 1683-1709

Serious diseases and pests of grass seed crops in the Czech Republic

Bohumír Cagaš

OSEVA PRO - Grassland Research Station, 756 54 Zubøí, Czech Republic

ABSTRACT

Based on the 1994 survey the acreage of grasses grown for seed in the Czech Republic equals about 11,000 ha. Permanent reproduction of foreign and native varieties of 23 grass species is accomplished, the most important species being meadow fescue (20%), annual ryegrass (16,9%), perennial ryegrass (15,7%), timothy (12%), Italian ryegrass 10,9%), red fescue (7,8%), and Kentucky bluegrass (4,4%).

The Grassland Research Station in Ro nov-Zubøí has been doing long-term multi-site observations and analyses of seed samples collected from the cleaning stations to get a real picture to formulate priorities in chemical pest and disease control as well as resistance breeding strategies.

Silver top caused by the bug Leptopterna dolobrata is controlled regularly in some species. Systemic fungicides are applied in Kentucky bluegrass to control rusts, powdery mildew and ergot in species cases. In recent year, mice have become a major problem in Lolium multiflorum. It seems necessary to initiate breeding for resistance to leaf spot (Drechslera poae), rusts (Puccinia brachypodii, Puccinia poarum), and powdery mildew in Kentucky bluegrass as well as to Drechslera dictyoides in meadow fescue.

Keywords: grass seed crops, long-term estimation, diseases, pests

INTRODUCTION

At present, grass seed are grown in the Czech Republic on the acreage of 11.000 hectares. Grass seed production has had a long tradition closely linked with the foundation of the Grassland Research Station at Ro nov pod Radhoštìm in 1920, and its subsequent successful breeding, seed production and advisory services. In recent years,

subsequent successful breeding, seed production and advisory services. In recent years, grass seed production can be characterized by a vast range of cultivated species (i.e. 22 species), a greater proportion of foreign cultivars, especially in ryegrass (more than 50% of the total acreage), and least but not last more and more phytopathological problems to be solved due to weeds, animal pests and/or fungus diseases. Based on the acreages, the most important grass species are as follows: meadow fescue (20%), annual ryegrass (16,9%), perennial ryegrass (15,7%), timothy grass (12%) Italian ryegrass (10,9%), red fescue (7,8%), and Kentucky bluegrass (4,4%).

METHODS

Basic data on serious infestations of the respective grass species in the major growing regions are available from the Grassland Research Station of Zubøí (the Czech Republic). One of the keyroles of the Station is to carry out analyses of the harvested seed lots that are further supplied to the specialized seed cleaning centre at Ro nov. Most of the serious phytopathological problems (ergot, rusts, silver top etc.) are being solved by the Station´s staff under the auspices of the Czech Grant Agency, National Agency for Agricultural Research, and the Ministry of Agriculture. The Station provides advisory and extension services in the sphere of grass seed production.

RESULTS

The analysis on the occurrence of seed grass diseases and pests in the Czech Republic accomplished in the last two decades has brought numerous results.

Ergot (Claviceps purpurea /Fr./Tul.) is the most serious fungus disease in Kentucky bluegrass. The recommended chemical control is not fully efficient. Substantial economic damage to Kentucky bluegrass seed production can be caused by leaf spot due to the fungus Drechslera poae Baudys., and rusts Puccinia brachypodii Otth var. poae nemoralis (Otth) Cummins et Greene, and Puccinia poarum Niels. Crested dog´s tail suffers from heavy winter loss due to fungal pathogens belonging to the genera Typhula. Out of insect pests, meadow plant bug (Leptopterna dolobrata L.) is in the limelight causing parasitary silver top (white head) in the genera Poa, Festuca, Agrostis, and Trisetum. Insecticidal control has become a common cultural practice. In recent years, there has been a spread of mouse rodents, particularly in Italian ryegrass.

Table 1 gives a survey of economically important and common diseases occurring in the cultivated grass species across the country.

DISCUSSION

At present, ergot is most probably the major fungus disease impairing seed yields and quality, particularly in Kentucky bluegrass due to the lack of reliable control means. In view of relatively high prices of systemic fungicides it is recommended to introduce post-harvest burning apart from fungicidal control in prebasic and basic seed production of Kentucky bluegrass in spite of possible problems burning can make under conditions of the Czech Republic. Consequently, there have been long-term efforts to elaborate a method of searching for donors of resistance to ergot. Based on our investigations (CAGAŠ 1995) it may be alleged that there are definitely some differences among host genotypes as well as pathogen isolates. Similarly, the experimental treatment of Kentucky bluegrass seeds infested with ergot sclerotia gave promising results (CAGAŠ 1992).

Graminicolous rusts, with the most frequent representatives Puccinia coronata Corda var. coronata, and Puccinia graminis subsp. graminicola Urb., are not of a great importance in mid- and east-European countries contrary to e.g. Belgium and the Netherlands (REHEUL and CHESQUIÉRE 1994). Normally, there is a vast spread of the rusts after seed harvest in the month of August, especially in perennial ryegrass, Italian ryegrass, and meadow fescue.

Kentucky bluegrass seed yield and quality can be adversely affected by Puccinia brachypodii Otth. var. poae-nemoralis (Otth) Cummins et Greene, and P. poarum Niels. that occur before heading. It is advised to control them by a systemic fungicide (CAGAŠ 1993 etc.) as well as to search for suitable sources of resistance among wild ecotypes. The same applies for powdery mildew.

Similarly, leaf spots caused by the fungi of the genus Drechslera are widespread across the Czech Republic, particularly Drechslera poae (Baudys) Shoem. plays an important role under cold and wet conditions, thus impairing meadow fescue seed yields. Until present, no chemical control has been taken into consideration which again emphasized the importance of searching for suitable donors of resistance. On the other hand, Drechslera dictyoides Shoem, and Drechslera siccans Shoem are less dangerous for seed yields of meadow fescue and perennial ryegrass. It is supposed to make a thorough

search to find out sources of resistance to be used in future resistance breeding programmes.

Powdery mildew (Erysiphe graminis D.C.) together with rusts can lead to premature leafdrop in seed plantations of Kentucky bluegrass an red fescue resulting in the emergency seed maturation. Fungicidal control is fairly efficient, if applied immediately upon the outburst of the disease.

Winter loss of grass seed stands, especially in Italian ryegrass due to the adverse combination of biotic and abiotic factors is quite common, but not very frequent, particularly upon condition that sowing dates are respected and plant stand is cut thoroughly before the winter. Plant stands of crested dog´s tail can be severely damaged by the fungi of the genus Typhula after the first seeding year. Nevertheless, this is a marginal problem because of small acreages of the above grass species.

Out of insect pest, there is much spread of meadow plant bug (Leptopterna dolobrata L.) causing the so called total white-head that occurs above all in the species of the genera Festuca, Poa, and Trisetum, but in recent years it has been attacking other species, e.g. perennial ryegrass. The infestation is typical for older plant stands (above one year), insecticidal control is completely inevitable to prevent from a considerable economic loss. In the 60s and 70s this disease was a major obstacle to growing meadow fescue and yellow oat grass in the former Czechoslovakia. Similar data have been reported in Hungary (BÜRGÉS, FISCHL and IVÁNY 1995). Partial silver top is a completely different disease attacking colonial bent grass, it is mainly caused by the members of the genera Oscinella, Haplothrips, and Aptinothrips with a possible share of the Fusarium species.

Mouse rodents have become a novel and serious phenomenon. They caused heavy damage to seed crop stands of Italian ryegrass, particularly in the years of 1994 and 1995. The problem of the appropriate control still remains unsolved.

Table 1 gives a list of other diseases that are more or less endemic, but under certain circumstances can get serious (e.g. choke in one timothy grass plantation in 1994, oat-grass nematode Anguina agrostis /Steinbuch/Filipjev in yellow oat-gras. It was also challenging to study the changes in the range and importance of the respective diseases occurring in the last two decades. Some diseases have become nearly extinct (e.g. Septoria oxyspora Penz. et Sacc.), whilst others have acquired novel importance, namely ergot and silver top.

Table 1

Diseases and pests in grass seed production in the Czech Republic (1975-1995)

Species	Acreage	Diseases/Pests	
	%	Economic importance	Other stresses
Agrostis stolonifera	0.4	*silver top*	
Agrostis tenuis	1.1	*silver top*	
Alopecurus pratensis	0.5		*Mastigosporium album*
Arrhenatherum elatius	1.6		*Ustilago perennans*
Cynosurus cristatus	0.8	*Typhula spp.*	
Dactylis glomerata	2.9		*Mastigosporium rubric.*
Deschampsia caespitosa	0.1		*Puccinia graminis*
Festuca arundinacea	0.1		
Festuca ovina	0.7		*Claviceps purpurea*
Festuca pratensis	20.0	*silver top* *Drechslera dictyoides*	*Puccinia coronata*
Festuca rubra	7.8	*Claviceps purpurea* *Erysiphe graminis*	
Lolium mult. subsp. ital.	10.9	*mice*	*Gerlachia nivalis*
Lolium mult. var. westerw.	16.9		
Lolium hybridum	0.0		
Lolium perenne	15.7		
Lolium x Festuca	1.3		
Phleum hubbardii	0.4		*Heterosporium phlei*
Phleum pratense	12.0		*Epichloe typhina* *Heterosporium phlei*
Poa compressa	0.0		
Poa nemoralis	0.2		
Poa pratensis	4.4	*silver top* *Claviceps purpurea* *Puccinia brachypodii* *Puccinia poarum* *Drechslera poae*	
Poa palustris	0.1		
Trisetum flavescens	2.1	*silver top*	*Septoria oxyspora* *Anguina agrostis*

The future global strategy for pest and disease control in seed grasses should be presumably focused on searching for novel sources of resistance to the various biotic stress factors to be incorporated into breeding programmes, even though there are still numerous problems now to match a high level of resistance with high yields. It is recommended to combine direct chemical control with phytosanitary measure.

REFERENCES

BÜRGÉS,G., FISCHL,G., IVÁNY,K. (1995): Investigation the reasons for white-spikedness at Kentucky Bluegrass (Poa pratensis L.) grown for seed. /Claviceps purpurea (Fr.) Tul./. Book of Abstracts, Third International Herbage Seed Conference, June 18-23, Halle (Saale), p.96.

CAGAŠ,B. (1993): Suppression of germination of ergot sclerotia /Claviceps purpurea (Fr.) Tul./ in grass seed by fungicidal treatment. Ochr. Rostl. 28:177-183.

CAGAŠ,B. (1993): Seed Yield and Diseases in Kentucky Bluegrass After Fungicide Applications. Journal of Applied Seed production 10: 11-14.

CAGAŠ,B. (1995): The resistance of Kentucky bluegrass (Poa pratensis L.) to ergot /Claviceps purpurea (Fr.) Tul/. Book of Abstracts, Third International Herbage Seed Conference, June 18-23, Halle (Saale), p. 39.

REHEUL,D., CHESQUIÉRE,A. (1994): Progress in Forage Grass Breeding. Symposium Agribex 94, Brussels, Acta Horticulturae 355: 135-141.

Diseases of *Deschampsia caespitosa* in sun and shade conditions

PRONCZUK M[a]., PRONCZUK S[a]., SADOWSKI CZ[b].

[a] Plant Breeding and Acclimatization Institute, Radzików, Poland
[b] Technical-Agricultural University Kordeckiego Bydgoszcz, Poland

ABSTRACT

During 1992-1995 the diseases of *Deschampsia caespitosa* ecotypes in comparison to other species of grasses were established in Poland. Ecotypes grown in different conditions: in nature, in the field for seeds, and on lawns in sun and shade. It was found that environmental conditions play the main role in disease occurrence on *Deschampsia caespitosa*. In the field *D. caespitosa* grown for seeds was susceptible to rust. *Puccinia graminis* was the causal agent of rust. In the sunny lawn *D.caespitosa* was moderately susceptible to leaf spot, rust, brown patch and fairy rings. In the shaded lawn and in natural sites only slight infection by diseases were observed. *Deschampsia caespitosa* ecotype "Brok" was the most healthy grass in shade conditions as compared to other common lawn species.

Keywords: *Deschampsia caespitosa*, fungal diseases, lawn grasses, sun and shade conditions.

INTRODUCTION

Deschampsia caespitosa is known to be a common weed grass on pastures. Some ecotypes are also found in the forest. Breeders have recently used this species for lawns (Oseva 1981, Pro czuk 1993, Barenbrug 1995). First variety "Meta" was registered in Czechoslovakia in 1981. In Poland, breeding program for *D. caespitosa* - with special emphasis on shade tolerance - started in 1988 (Pro czuk 1993, 1994).

Shade creates unfavorable conditions for most species of grasses and usually diseases decrease the quality of the lawns. It was important to increase knowledge of the diseases of *Dechampsia caespitosa*.

The aim of this study was the evaluation of diseases prevalence on *Deschampsia caespitosa* ecotypes grown in different conditions: in nature, in the field for seeds, and on lawns in sun and shade. Special emphasis has been put on the ecotype "Brok" selected from the forest.

MATERIALS AND METHODS

Surveys of diseases on *D. caespitosa* ecotype grown in nature were done in five sites in the Bydgoszcz region during the years 1994-1995. In each site about 30 plants were assessed according to the disease symptoms.

38 ecotypes collected from different locations in Poland were grown for seeds in the field nursery at Radzików (central Poland). They were planted in a space of 40 x 40 cm with fertilization conditions of: 120 N, 120 K_20 and 60 P_20_5 kg/ha. The ecotypes were observed during 1995, and occurrence of diseases were evaluated visually .

The ecotype "Brok" collected from the forest after a pre-breeding test was included in lawn trials. Two following trials were done in sun and shade conditions:

I - 1992-1994 - for comparisons of *Deschampsia caespitosa* ec. "Brok" to 5 common lawn species represented by 15 varieties:

Festuca rubra:	Leo, Nimba, Dawson
Festuca ovina:	Sima, Niko, KRH-11
Festuca arundinace:	Sinfonia, Bartes, Villagoise
Lolium perenne:	Bramka, Nira, Gazon
Poa pratensis:	Alicja, Gol, RAH-213

II - 1994-1995 - for comparison of *D. caespitosa* ec. "Brok" to *Poa pratensis* represented by the 7 following varieties:

Gol, Alicja, Limousine, Parade, Trampas, Haga, Sydsport.

The lawn trials were conducted at Radzików (central Poland) at an experimental field (sun) and at a nature park (shade). The sun lawn tests were done under medium high maintenance : 25-30 cuts, 3 cm high, fertilization level: 200-250 N, 120 K_20, 60 P_20_5 kg/ ha per year with irrigation during the summer. The shade lawn tests were done under the following low maintenance regime : 10-12 cuts, 5-7 cm high, fertilization: 60-80 N, 60 K_20, 30 P_20_5 kg/ha per year, without summer irrigation. The first trial was located in heavy shade and the second in medium shade.

Disease severity and the aesthetic aspect of the lawns were rated on a scale of 1-9 where 1= represented complete infection, poor appearance of the lawn, and 9= the absence of disease symptoms and excellent aesthetic aspects of the lawn.

The results are calculated for species using an average of the varieties data.

All data were summarized and subjected to variance analysis. The least significant difference (LSD) method was used for multiple comparisons of means.

RESULTS

Table 1 includes results of observations of *Deschampsia caespitosa* plants in the nature sites. The ecotypes of *D. caespitosa* appeared healthy. Only slight infections with leaf spot, powdery mildew, and rust were noticed. In nature no problem with diseases was found.

Table 1: Diseases of *Deschampsia caespitosa* ecotypes grown in nature sites in the Bydgoszcz region during 1994-1995.

Site	1994			1995		
	Leaf spot	Powdery mildew	Rust	Leaf spot	Powdery mildew	Rust
I	8.3	8.2	8.1	8.1	8.3	8.0
II	8.5	8.5	8.2	7.9	8.2	7.9
III	8.2	8.0	8.2	8.2	8.0	8.2
IV	8.2	8.5	8.1	8.1	7.9	8.3
V	8.3	8.4	8.0	8.2	8.2	8.2

Rating scale: 1-9, where 1 = complete infection, and 9 = absence of disease symptoms.

However ecotypes of *D. caespitosa* collected from nature sites were very susceptible to rust in the field conditions. Table 2. contains data of the estimation of rust infections of *D. caespitosa* ecotypes in the field nursery in Radzików in 1995. Infection of rust came after harvesting time in August. In September leaves of the majority of ecotypes were completely destroyed by rust. It was found that *Puccinia graminis* was the main infectious leaf agent of *D. caespitosa.*

Table 2: Rust infection on *Deschampsia caespitosa* ecotypes collected in the field at Radzików in 1995.

Site of origin	Site conditions	Number of ecotypes	Level of rust infection
Brok	shade	10	3-5
Belchatów	sun	3	2-3
Wolsztyn	shade	1	2
Jaroslaw	shade	3	2-8
Lopuszna	sun	5	2-5
Cieszyn	shade	5	2-4
Zelazowa Wola	shade	4	2-9
Sieniawa	shade	1	7
Laski	shade	1	3
Meta (variety)	?	5	3-8

Rating scale: 1-9, where 9 - absence of symptoms

The results of the estimation of *D. caespitosa* diseases in comparison to five species of grasses in sunny and shady lawn conditions are presented in table 3. *Deschampsia caespitosa* generally performed the best in comparison to other species in the shady. In sun we have found some problems with leaf spot (*D. triseptata*), rust (*P. graminis*), and fairy rings. The main diseases of other grasses in experiments were: leaf spot, powdery mildew, rust, snow mould and fairy rings. Powdery mildew (*Erisiphe graminis*) was observed only in the shade, particularly on *Poa pratensis,* on the contrary, rust (*Puccinia poae nemoralis*, *Puccinia gramins*) and fairy rings which were found only in sun. In both conditions the grasses were injured by leaf spot (*Drechslera poae, D.*

dictioides, *D. triseptata*) and snow mould (*Microdochium nivale*). Injury by diseases reflects on the aesthetic aspect of lawns.

Table 3. Diseases and aesthetic aspects of *Deschampsia caespitosa* ec. "Brok" in comparison to other species of grasses in sun and shade lawn conditions, Radzików 1992-1994.

Species	Disease severity										Aesthetic aspects of lawn	
	Leaf spot		Powdery mildew		Rust		Snow mould		Fairy rings			
conditions	sun	shade	sun	shade	sun	shade	sun	shade	sun	shade	sun	shade
observation n=	12	12	12	12	12	12	12	12	6	6	27	27
Deschamp. caespitosa	6.7	8.7	9.0	8.7	7.0	9.0	9.0	8.3	++		6.6	7.4
Festuca rubra	7.2	8.1	9.0	8.8	8.9	9.0	5.2	6.8	+		6.8	6.0
Festuca ovina	7.1	8.5	9.0	8.8	8.8	9.0	5.6	5.9			5.3	5.5
Festuca arundinace	6.9	8.0	9.0	8.6	9.0	9.0	3.7	6.4			6.4	6.8
Lolium perenne	7.5	8.4	9.0	8.3	9.0	9.0	4.6	7.2	+		6.9	6.0
Poa pratensis	5.7	6.3	9.0	5.0	7.3	9.0	9.0	7.5	+		6.7	3.7
LSD 0.05	0.5	0.8		0.7	0.5		0.6	1.9			0.5	0.7

Rating scale: 1-9, where 9= absence of disease symptoms and the best aesthetic aspect of lawn.

In table 4 are data from the trials of lawn in comparisons of *D.caespitosa* ecotype "Brok" to *Poa pratensis* varieties. The results follow a similar trend as that established in the previous experiments. *D. caespitosa* occupied the top rankings in shade. However in sun conditions Brok was susceptible to brown patch (*Rhizoctonia solani*). This disease

reduced the aesthetic aspects of lawn during infections but it did not seem to cause any long term damage. *Poa pratensis* varieties did not perform well because they were susceptible to powdery mildew (*Erisiphe graminis*) in shade and to leaf spot (*Drechslera poae*) in both conditions. Compared varieties of *Poa pratensis* expreseed a wide range of susceptibility to diseases (from 4.1 to 6.8). Varieties Trampas and Limousine demonstrated better resistance to leaf spot and powdery mildew and aesthetic aspects of lawn appeared better than other varieties of *Poa pratensis*.

Table 4. Diseases and aesthetic aspects of lawn of *Dechampsia caespitosa* ec."Brok" in comparison to *Poa pratensis* varieties in sun and shade conditions, Radzików 1994-1995.

Varieties	Disease severity								Asthetic aspects of lawn	
	Leaf spot		Powdery mildew		Rust		Brown patch			
conditions	sun	shade	sun	shade	sun	shade	sun	shade	sun	shade
observ. n=	6	6	6	6	6	6	6	6	18	18
D. caespitosa Brok	7.5	7.8	9.0	9.0	8.3	8.8	6.0	9.0	6.2	8.3
P.pratensis Gol	6.1	5.3	9.0	4.1	6.3	7.0	9.0	9.0	6.3	4.9
Alicja	4.6	4.9	9,0	6.2	7.0	6.6	9.0	9.0	6.4	4.8
Limousine	6.8	6.8	9.0	6.0	6.6	6.6	9.0	9.0	7.4	6.5
Parade	5.1	4.6	9.0	5.1	7.0	7.8	9.0	9.0	6.0	4.7
Trampas	6.1	6.3	9.0	6.8	8.0	8.3	9.0	9.0	6.9	7.2
Haga	4.5	5.0	9.0	5.8	7.0	7.8	9.0	9.0	5.9	5.0
Sydsport	5.6	5.8	9.0	5.8	7.6	7.8	9.0	9.0	5.8	6.3
LSD 0.05	0.6	0.8		0.6	0.9	0.7	0.5		0.5	0.5

Rating scale: 1-9, where 9 - absence of disease symptoms and the best aesthetic aspect of lawn

DISCUSSION

Results of this study indicated that environmental factors and maintenance influenced the development of diseases on *D.caespitosa* plants. Single ecotypes grown in natural sites appeared healthy. However change of growth place from natural to field conditions revealed high susceptibility to rust. According to Smiley et al., (1993) rust diseases become most severe on grasses that are growing slowly under stressful conditions. Typical stress is drought. In August 1995 drought was observed on the experimental field in Radzików and it could influence on severe injury of *D. caespitosa* leaves. Stem rust occurs commonly in *Lolium perenne* and *Festuca arundinaceae* and infects usually culms and spikeletes of the host (Welty and Azevedo 1995, Kulik and Dery 1995). On *D. caespitosa* ecotypes were effected only leaves.

Results from lawn trials showed that *D. caespitosa* ec. "Brok" is more healthy grass than other varieties in compared species, particularly in shade conditions under low maintenance. Many reports related that shade conditions are unfavorable for grasses (Funk 1981, ASTRI 1987). The microclimate of the shaded area favors the pathogens to develop diseases (Baldwin 1990, Smiley and et al., 1993). For example the grass growing in the shade or around which there is poor air circulation may be more severely damaged by powdery mildew (Baldwin 1990). This disease has been very important in shade on *Poa pratensis*. However on *D. caespitosa* plants no injury by *Erisiphe graminis* were noticed.

Common disease of lawn was snow mould caused by *Microdochium nivale*. Some species of grasses like *Lolium perenne*, *Festuca* spp. were very susceptible to this disease. The ecotype of *D.caespitosa* appeared to be resistant to snow mould.

Leaf spot diseases caused by *Drechslera* spp. fungi were observed common on lawn grasses. Occasionally the disease resulted in serious damage on *Poa pratensis*. That stage is often referred to as "melting out" (Smith et al., 1994). Leaf spot disease on *D. caespitosa* plants occurred only on the top of leaves. Infections by *Drechlera triseptata* and accompanied fungi like *Alternaria* sp, *Cladosporium* sp. were not severe and might connect with mower injury.

Important disease of *D. caespitosa* was brown patch caused by *Rhizoctonia solni*. Damage of lawn were not severe but laboratory tests showed high pathogenicity of isolates *Rhizoctonia solani* and *Rhizoctonia zeae* (Pro czuk 1995). This suggests that

brown patch may be severe disease for *D. caespitosa* plants if local and seasonal conditions would favor the development of the fungus. According to Burpee (1995) initial symptoms of brow patch are usually observed after several consecutive nights with minimum temperatures>16°C and leaf wetness periods>10h. That conditions sometimes occur in Poland.

In sun conditions on the lawn of *D.caespitosa* fairy rings was observed. They were very common and usually the rings were with fruiting bodies without apparent effect on the *D. caespitosa* plants. Fairy rings have been classified into 3 types according to the effect on the grasses (Baldwin 1990) . Rings on *D. caespitosa* lawn was identified as type 3. This type is known to be of low severity for grasses.

CONCLUSIONS

1. The environmental conditions play the main role in disease occurrence on *Deschampsia caespitosa*:
   - in the field grown for seeds was susceptible to rust. *Puccinia graminis* was the causal agent of rust,
   - in the sunny lawn was moderately susceptible to leaf spot (*Drechslera* spp.), rust (*Puccinia graminis*), brown patch (*Rhizoctonia* spp.) and fairy rings,
   - in the shaded lawn and in natural sites only slight infection by diseases were observed.
2. *Deschampsia caespitosa* ecotype "Brok" was the most healthy grass in shade conditions as compared to other common lawn species.

RFERENCES

ASTRI. 1987. Grass in Shade. Sports Turf Bulletin 159 : 9-11.

Baldwin N. A., 1990. Turfgrass pests and diseases. Sports Turf Research Institute : pp. 49

Barenbrrug 1995. Range of varieties. Bulletin: 15.

Burpee L. L. 1995. Interactions among mowing height, nitrogen fertility, and cultivar after the severity of Rhizoctonia blight of tall fescue. Plant Dis. 79:721-726.

Funk C. R., 1981. Perspectives in turfgrass breeding and evaluation. Proc. of tht 4th Inter. Turfgrass Research Conf. July 19-23 Ontario, Canada: 3-10.

Kulik M. M., Dery P. D. 1995 The infection of *Festuca arundinacea* by *Puccinia graminis* subsp. *graminicola*. Jurnal of Phytopat. 143(1): 53-58.

Oseva - Information, 1981. Meta. Oseva lechtitelsky ustav picninarsky Troubsko u Brna, Czech Republic.

Pronczuk S.,1993. *Deschampsia caespitosa* L. - Proposal for lawn in shade conditions. Abstracts of the 18th Eucarpia "Breeding for marginal conditions". Fodder crop section meeting 25-28 August, Leon, Norway: 8.

Pronczuk S., Pronczuk M., 1994. Looking for shade tolerance in lawn grasses. Proceedings of the 19th Eucarpia "Breeding for quality". Fodder crops section meeting 5-8 October Brugge, Belgium: 77-81.

Pronczuk M., Pronczuk S. 1995. Occurrence of brown patch disease on turfgrasses in Poland. Abstracts of the International Symposium on Rhizoctonia "Facts and Challenges in Pathology, Taxonomy, Ecology and Disease Control" 27-30 June Noordwijkerhout, the Netherlands: 72.

Smiley R. W., Dernoeden P. H., Clarke B. B. 1993. Compendium of Turfgrass diseases. American Phytopath. Society ; pp 98

Smith D. A., Bara R. F., Dickson W. K., Clarke B. B., Funk C. R. 1994. Leaf spot on Kentucky Bluegrass cultivar evaluation trials at Rutgers University. Rutgers turfgrass Proc. of the New Jersey Turfgrass Expo December 7-9, 1993, Atlantic City: 116- 138.

Welty R. E., Azevedo M. D. 1995. Occurrence of *Puccinia graminis* subsp. *graminicol* in Chewing fescue in Oregon. Plant Dis. 79:1014-1016.

FURTHER INVESTIGATIONS ON THE HOST RANGE OF *XANTHOMONAS CAMPESTRIS* PV. *POAE*

Dorothea Schmidt

Station fédérale de recherches agronomiques de Changins,
Case postale 254, CH-1260 Nyon 1, SWITZERLAND

Abstract

In the first paper on *X.c.*pv. *poae* (Egli and Schmidt, 1982) the host range of these bacteria seemed restricted to the genus *Poa*, except for a few wilting *Lolium* plants. *Poa pratensis* was considered highly resistant. Since that publication *X.c.*pv *poae* has been isolated from naturally infected *Poa pratensis* plants in Germany (Birckenstaedt et al., 1994) and from wilting *Bromus sitchensis* found in France. Recent inoculation tests indicate that some selected clones out of 2 commercial cultivars of *Poa pratensis* produced very susceptible progenies, showing a higher mortality rate than the commercial seed of these cultivars. Some cultivars of *B. sitchensis* and *B. catharticus* were found to be as susceptible to infection by *X.c.*pv. *poae* as by *X. bromi.* We conclude from these experiments that the host range of *X.c.* pv. *poae* is larger than previously anticipated : it comprises also certain genotypes of *Poa pratensis* and of *Bromus* spp.

Keywords : *Xanthomonas campestris* pv. *poae*, *X. bromi*, host-range, *Poa pratensis*, *Bromus* spp.

Introduction

For plant pathologists and plant breeders it is important to know the host range of phytopathogens, in order to choose the right strains for resistance screening and in order to understand epidemiological observations.

In their paper describing 4 grass-inhabiting pathovars of *X. campestris* Egli and Schmidt (1982) proposed a discrimination set of host plants (tab.1).

X.c. pv. *graminis* has a rather large host range, including all *Lolium* spp. and *Festuca* spp., *Dactylis glomerata, Trisetum flavescens* and others. *Phleum pratense* and *Poa trivialis* can be infected, however without serious consequences to the crop. *X.c.*pv. *phlei* was restricted to the genus *Phleum* and *X.c.*pv. *arrhenatheri* to *Arrhenatherum elatius*. *X.c.* pv. *poae* was restricted to the genus *Poa,* however a small percentage of *L. multiflorum* plants often showed severe wilting when inoculated with pv. *poae.*

It was soon evident that more or less resistant or susceptible genotypes of a same plant species exist. Thus a highly susceptible variety should be used to check the pathogenicity of bacterial isolates.

Table 1 Usual response pattern of a standard set of grasses to four pathovars of *X. campestris* (Egli and Schmidt, 1982)

Set of differential host plants	***Xanthomonas campestris***			
	pathovar *graminis*	**pathovar *phlei***	**pathovar *arrhenath.***	**pathovar *poae***
Lolium multiflorum	+++	-	-	- (+)
Phleum pratense	+	+++	-	-
Arrhenatherum eliatus	-	-	+++	-
Poa trivialis	+	-	-	+++

Table 2 shows the observed susceptibiltiy of *Poa* spp. to *X.c.*pv. *poae*. *Poa trivialis* is most susceptible, it is the main host plant in nature. *Poa pratensis* was considered highly resistant. Cultivar Newport C1 was used at that time, but other cultivars and ecotypes were tested too.

Table 2 Response of six *Poa spp. to X.c.* pv. *poae* (Egli and Schmidt, 1982)

Poa trivialis		highly susceptible
Poa nemoralis	}	susceptible
Poa fertilis		
Poa annua		little susceptible
Poa compressa	}	highly resistant
Poa pratensis		

Birckenstaedt et al. (1994) reported having isolated *Xanthomonas* bacteria from *Poa pratensis* cv. Erte in Germany, which grouped together with isolates from *Lolium* and *Bromus* in a dendrogram of average linkage for disease scores, fatty acid patterns and phenotypic aspects.

One of our isolates from diseased Bromus plants from France turned out to be *X.c.* pv. *poae*.

Experimentation

These two new findings encouraged us to carry out a new host range trial for *X.c.* pv. *poae*.

A strain of the *Poa*-isolates from Birckenstaedt (Xcpo2) was confirmed to be *X.c.*pv.*poae*, inducing however a little less mortality on *P. trivialis* than our highly pathogenic strain 724 (table 3). *P. pratensis* cv. Erte seems to be highly resistant to bacterial wilt in this inoculation trial.

Table 3 further indicates that 27 of the tested progenies from *Poa pratensis* clones selected in ecotypes from Switzerland and Southern Germany were highly resistant to bacterial wilt. However, progenies of 3 clones issued from reference cultivars in our breeding program were susceptible to *X.c.* pv. *poae* strain 724. Two of these clones originate from cv. Tommy and one from cv. Jori.

Table 3 reaction of *Poa pratensis* breeding lines to inoculation with three *Xanthomonas* isolates

Number of lines	Origine	% mortality when inoculated with *Xanthomonas campestris* pv.*poae* 724	pv.*poae* xcpo2	pv.*graminis* Xg 1/93
27	ecotypes CH and D	0 - 2	0 - 2	0
2	cv. Tommy (F)	39-84	5	0 - 13
1	cv. Jori (D)	44	8	12
Standard cultivars				
Poa pratensis	cv. Erte (Danemark)	0	4	0
Poa trivialis	commercial seed	88	50	0
Lolium multifl.	cv. Tewera	5	7	88

Table 4 shows the reaction of commercial seed of Tommy, Jori and some other cultivars in 2 inoculation experiments. Tommy developped 28% of mortality, less than the progenies of the two selected clones, and Jori was very little affected, as were all the other cultivars.

Table 4 inoculation of *Poa pratensis* cultivars with *Xanthomonas campestris* pv. *poae* 724

Poa pratensis cv.	% mortality	
	1st trial	2nd trial
Tommy	28	28
Jori	6	7
Leikra	3	0
Monopoly	7	0
Tendos	0	2
Sobra	11	1
Erte	n.t	4
experiment. cv. 743/94	15	6
experiment. cv. 712/93	9	1
Poa trivialis	92	93

This observation indicates that selection is possible to a certain extent in *Poa pratensis*, a species which is normally fully apomictic. Selection of single tillers can increase or decrease resistance to certain diseases.

Concerning the *Bromus* problem, several *Bromus* spp. and cultivars from hot, dry countries were introduced in France in the seventies and bred for forage purposes destinated to regions with occasional water stress. Some of these cultivars soon showed severe symptoms of bacterial wilt and had to be abandoned, e.g. *B. carinatus* Luval.

Two different *Xanthomonas* bacteria were isolated from diseased plants obtained from France in 1980 (table 5).

Table 5 reaction of 3 *Bromus* spp. to inoculation with 3 pathovars of *X. campestris*, % mortality

Plant species	***Xanthomonas campestris***			
	pv.*poae* ex-Lubro	**pv.*poae* 724**	**X.*bromi* ex-Luval**	**pv. *graminis* 1/93**
B. sitchensis cv. Lubro	0	0	75	0
B. catharticus cv. Broma	0	0	60	0
B. catharticus cv. Matua	31	96	90	0
Lol. multifl. cv. Tewera	0	0	0	93
Poa trivialis	77	91	0	25

The isolate from *B.sitchensis* cv. Lubro turned out to be *X.c.* pv. *poae*, slightly less agressive than strain 724. It is astonishing that in the experiment presented in tab. 5. Lubro did not show any mortality when inoculated with pv.*poae*. In a recent inoculation trial Lubro developped 55% of mortality when inoculated with strain 724. We cannot explain the difference; maybe environmental effects such as temperature can influence disease development.

The isolate from *B. carinatus* cv. Luval seems specific to *Bromus* spp. Based on analysis of similar isolates from France and New Zealand Vauterin et al. (1992) proposed to rank these bacteria to a species level, *X .bromi.* They tried to reorganise the *Xanthomonas* classification, in particular the species *X. campestris* to which almost 150

pathovars had been attributed and which is does not satisfy anyone. All grass-inhabiting Xanthomonads group together with those from cereals (translucens group) with DNA binding levels above 60%, except *X. bromi* and *X. oryzae.* However, all are quite distinct from *X. campestris.*

Experiments to establish the host range of *X. bromi* are presently undertaken by Mrs. R. Samson, INRA-Angers, M. Betin, INRA-Lusignan and D. Schmidt, RAC-Switzerland.

Some *B. catharticus* cultivars can be susceptible to both *X. bromi* and X.c. pv. *poae* (table 5).

Conclusion

We conclude from these experiments that the host range of *X.c.* pv. *poae* is larger than previously anticipated : it also comprises certain genotypes of *Poa pratensis* and of *Bromus* spp.

References

Birckenstaedt E., Hein D., Kettrup A., Paul V.H., Janse J.D., 1994. Taxonomic analysis of *Xanthomonas campestris* pv. *graminis, X.c.* pv. *arrhenatheri*, *X.c.* pv. *poae* and *X.c.* pv. *phlei* by means of pytopathological , phenotypic and fatty acid analysis methods. IOBC wprs Bulletin, 17: 45-57

Egli T. and Schmidt D., 1982. Pathogenic variation among causal agents of bacterial wilt of forage grasses. Phytopath. Z. 104 : 138-150

Vauterin L., Yang P., Hoste B., Pot B., Swings J., and Kersters K, 1992. Taxonomy of xanthomonads from cereals and grasses based on SDS-PAGE of proteins, fatty acid analysis and DNA hybridization. J. of General Microbiology 138 : 1467-1477.

Symbiosis effects between *Festuca* and *Acremonium* species in single plants

PFANNMÖLLER, MONIKA; EGGESTEIN, ST.; SCHÖBERLEIN, W.

Institut für Acker-und Pflanzenbau, Saatgutwirtschaft, Landwirtschaftliche Fakultät der Martin-Luther-Universität Halle-Wittenberg, Germany

ABSTRACT

A field trial with endophyte-infested and endophyte-free meadow and tall fescue was established to determine the endophyte influence on tiller number per single plant and seed yield and to study the dynamics of endophyte infection over three years.
The results led to the conclusion that there is a trend to higher numbers of fertile tillers in endophyte-infested *F. pratensis* varieties. The seed yield increased in the E^+-meadow fescue varieties. From 1993 to 1995 the endopyte level decreased varyingly. This behaviour seemed to have been caused by the different aptitude of the genotype to the association with *Acremonium.*

Keywords: endophyte, *Festuca pratensis, F. arundinaceae*, single plants, field trial, seed yield

INTRODUCTION

Several activities have been launched to study the influence of endophytic fungi on the vegetative and generative development of ryegrass and tall fescue. So far, only some investigations by SCHMIDT (1991 and 1994) and BUMERL et MIKA (1991) have been published about meadow fescue which in Europe outrans tall fescue in fodder grass production. So we tried to evaluate the influence of *Acremonium uncinatum* on single plants of two meadow fescue varieties and additionally that of *Acremonium coenophialum* on a tall fescue variety. The field trial was established to determine the endophyte influence on the number of tillers per single plant and the seed yield as well as the endophyte infection of harvested seeds over three years.

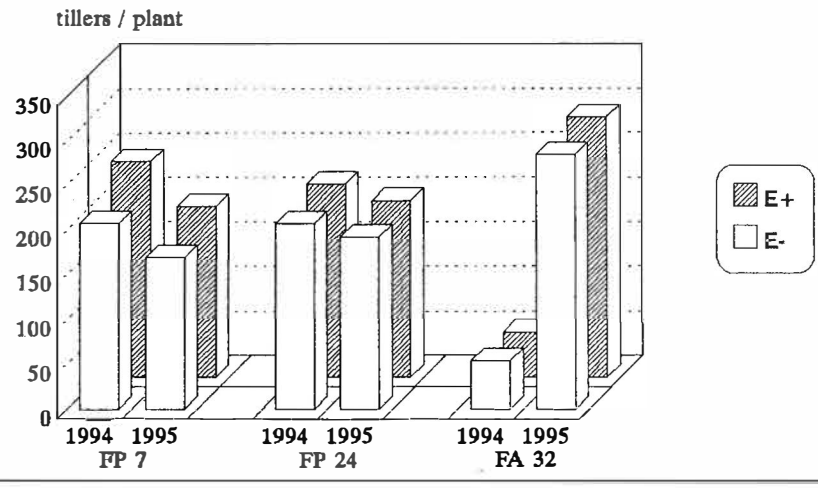
Field emergence was not taken into consideration. This is different from drilled trials where the seed yield reflects rather field emergence and number of plants per plot than the capacity of the plant to produce more seeds.

MATERIALS AND METHODS

Two *Festuca pratensis* varieties (FP 7 and FP 24) and one *F. arundinacea*-variety (FA 32) which were known to harbour endophytic fungi were used. The seeds were sown in plastic boxes in January and February 1993. 6-12 weeks later the plantlets were microsopically screened to divide the populations into endophyte-infested (E^+) and endophyte-free (E-) plants. In June 1993 we planted the young plants out into the field at the experimental station of Halle university. A randomized block design with 21 plants in each plot and 4 replications (altogether 84 plants in each variant) was used.

In 1994 and 1995 the examined parameters included:
- number of fertile tillers
- seed yield
- general impression before winter
- assesment of diseases
- endophyte level in harvested seeds.

Figure 1: Fertile tiller number of two meadow fescue and one tall fescue varieties in a single plant trial

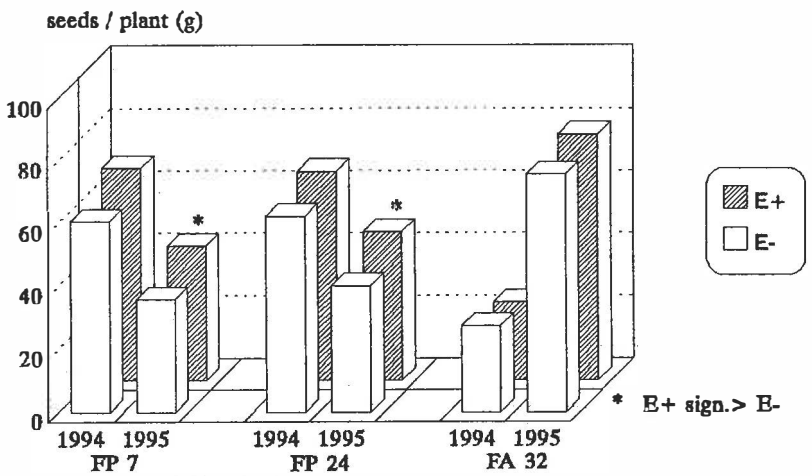


RESULTS AND DISCUSSION

Between 1994 and 1995 the development of the plants, especially those in the tall fescue plots, showed a great variation (Figure 1). In the first year the number of fertile tillers was smaller than in 1995. Tall fescue revealed no variation in the tiller number between E^+ and E- variants. In the first and second year the two meadow fescue varieties produced a high number of fertile tillers. In both years the E+ meadow fescue variants developed slightly more tillers, however the differences were not significant.

The tall fescue seed yield showed a similar picture (Figure 2) and reached only approximately 25 g per plant in 1994 whereas in 1995 we harvested more than 75 g/plant. The results varied little, but not significantly. Another result became obvious for the meadow fescue varieties. In all cases the E^+ plots reached higher seed yields of 11 and 7% in 1994 and 15 and 20% in 1995 compared to the E- plots. The results of 1995 were significant for both meadow fescue varieties.

Figure 2: Seed yield of two meadow fescue and one tall fescue varieties in a single plant trial



In some cases the seed yield per tiller was similar in the endophyte- infected and endophyte-free variants, a slightly higher seed yield per tiller in favour of the E^+ plants of the meadow fescue varieties was achieved in 1995. The evaluation of the general

impression before winter showed that particularly in 1995 the E^+ variants looked much better than the E- plots.

In 1994 the estimation of plant diseases on the tall fescue plants showed a slight, in 1995 a strong infection by *Puccinia coronata*, but no influence by the endophyte status on crown rust infection could be discovered. There was no crown rust infection of the neihbouring meadow fescue plants at that moment. No other diseases were discovered in the investigated period.

After the harvest the endophyte status in the harvested seeds was checked. The principle of this examination is shown in Table 1.

Table 1: Development of the endophyte level in a single plant trial with meadow and tall fescue

Time	Number of seeds or plants examined	**Variety FP 7**				**Variety FA 32**			
January 1993	100 seeds	seed lot with 23 % E^+				E^+ seed lot			E^- seed lot
March 1993	200 plantlets	84 E^+ plants			84 E^- plants	84 E^+ plants			84 E^- plants
August 1994	5 seeds from all plants	74 E^+ plants			82 E^- plants	67 E^+ plants			79 E^- plants
October 1995	10 seeds from 14 plants	14 plants			3 plants	14 plants			3 plants
		10 E^+	2 medium	2 E^-	3 E^-	5 E^+	2 medium	7 E^-	3 E^-

The variety FP 7 comprises one seed lot with medium endophyte infection level. In January 1993 100 seeds were examined, and the infection level was found to be 23%. After germination and growing for six weeks all plants were microscpically screened and the population divided into E^+ and E- plants. These 84 plants were used for the field trial. After the seed harvest 1994 five seeds from each plant were examined to determine the endophyte status. The results revealed that not all plants marked as endophyte-positive contained endophytes after the first examination, and not all endophyte-negative samples were actually endophyte-free. The scheme shows that from the 84 E^+ plants of FP 7 as

used in the field trial only 74 plants had produced endophyte-positive seeds. This may be due to the fact that the endophyte infection was not stable. On the other hand, 82 of 84 E--plants produced endophyte-free seeds. The other meadow fescue variety FP 24 showed a similar behaviour. In the tll fescue variety FA 32 the number of infested plants decreased more than in the meadow fescue varieties.

In 1995 we made a second examination of the harvested seeds. The results were unexpected. 10 seeds from 14 plants endophyte-positive in 1994 and 10 seeds from 3 endophyte-negative plants were examined. From the 14 checked FP 7 plants only 10 plants produced seeds which were all E^+, 2 plants seemed to have lost the endophytes, two had an medium infection range (90, 92%) in the seeds.
We got a similar result for the FP 24 variety: 5 plants produced only E^+ seeds, no plant was found to be endophyte-free and 9 had an intermediate level ranging from 10 to 90% in the seed. The results with tall fescue were more surprising: only 5 priviously E^+ labelled plants were found to have all E^+ seeds, 7 had lost the endophyte and 2 had a level of 50 and 75%.

In a previous study (BUMERL and MIKA 1991) the conclusion was drawn that some seeds in the panicle or even all seeds in some panicles, were free from infection. The mycelium growth throughout the plant may be slowed down by the interference of a number of external and internal factors that do not affect the normal growth of the tiller. These tillers may than outgrow the fungus, especially under conditions less favourable for endophyte growth.

To get further results the plants which had previously contained endophytes and the seeds thereof now revealed no infection were examined. Our results showed that all 7 tall fescue plants which had E- seeds were not endophyte-infested. This fact allowed to draw the conclusion that the plant had lost the endophyte. Perhaps the genotype of the endophyte and the genotype of a particular plant did not allow the fungus to grow, therefore that the infection became instable.

A summery of the development of the endophyte content in all three varieties is given in the Table 2. During one year some plants lost the endophyte, in the case of FA 32 this referred to 50% of the plants. In FP 24 no former E^+ plant was found to be E-, but 65 % did not produce complete E^+ seeds. FP 7 is the most stable variety with 72 % E^+ plants

producing E^+ seeds. We got these results in the last few weeks, and now we have to find out the reasons for this behaviour.

Table 2: Development of the endophyte level from 1994 to 1995 in single plants

Variety	Endophyte status in the seeds of plants labelled as E^+ in 1994		
	complete E^+	medium	E^-
FP 7	72%	14%	14%
FP 24	35%	65%	0%
FA 32	36%	14%	50%

CONCLUSIONS

The results allow to draw the conclusions that there was a trend to higher fertile tiller numbers for the endophyte-infested *F. pratensis* varieties. The seed yield was higher in the E^+-meadow fescue varieties. The endopyte level decreased from 1993 to 1995 to a different degree. This behaviour could be caused by the different aptitude of the genotype for the association with *Acremonium.* In cross pollinated plants the effect of endophytes on some agricultural parameters is mixed with the diversity of these parameters in plant populations. To get additional results, further research is necessary with genetically homogeneous plants with and without endophytes.

REFERENCES

BUMERL, J. and MIKA, V. 1991: Infection of meadow fescue and *Festuca arundinaceae* by the fungus *Acremonium coenophialum:* The effect on seed yield and quality. Scientific studies, Oseva, Breeding Institute for fodder plants, Troubsko, 12, 187-191

SCHMIDT, D. 1991: Les endophytes de la fétuque des prés. Revue suisse Agric., 23 (6), 369-375

SCHMIDT, D. 1994: Du nouveau sur les endophytes de la fétuque des prés. Revue suisse Agric., 26 (1), 57-63

This work was supported by the Deutsche Forschungsgemeinschaft.

Effect of cutting height on perennial ryegrass with and without infection with endophyte and ryegrass mosaic virus

G.C. LEWIS

Institute of Grassland and Environmental Research, North Wyke Research Station, Okehampton, Devon EX20 2SB, UK

ABSTRACT

Plants of one genotype of perennial ryegrass (*Lolium perenne*) grown in pots in a glasshouse were harvested on six occasions over a 30-week period. Eight treatment combinations were applied, namely ± infection with the endophytic fungus *Acremonium lolii* (E) x ± infection with ryegrass mosaic virus (RMV) x two cutting heights (5 and 1 cm). The main effects of treatments were that the herbage dry matter yield accumulated over harvests 2-6 was 6.6% higher for +E plants than for -E ($P< 0.05$), 31.3% higher for -RMV plants than for +RMV ($P< 0.001$), and 106.2% higher for plants cut at 5 cm height than for those cut at 1 cm ($P< 0.001$). The accumulated yield for +E+V plants was 13.7% higher than -E+V ($P< 0.05$) at the 5 cm cut and 65.0% higher at the 1 cm cut ($P< 0.05$); there was no significant effect of E with -V plants. It is concluded that +E plants were more tolerant than -E of stress induced by RMV infection and severe defoliation.

Keywords: *Acremonium lolii*, *Lolium perenne*, ryegrass mosaic virus, herbage yield, cutting height

INTRODUCTION

In the UK, infection of *Lolium perenne* with ryegrass mosaic virus (RMV) is widespread and common, and reduces herbage yield (Jones *et al*, 1977). In a previous experiment (Lewis & Day, 1993) the reduction in yield of *L. perenne* infected with RMV and barley yellow dwarf virus was reduced significantly when the plants were also

infected with the endophytic fungus *Acremonium lolii*. This paper reports results of a glasshouse experiment in which plants of *L. perenne* with and without infection with RMV and *A. lolii* were harvested at two cutting heights on six occasions.

METHODS

All the plant material used in the experiment originated from one *A. lolii*-infected plant of *L. perenne* collected from a long-established sward in north-west England. This plant was split into ramets and some ramets were grown hydroponically to eradicate infection by *A. lolii* (Lewis & Vaughan, 1995). Some *A. lolii*-infected (+E) ramets and some *A. lolii*-free (-E) ramets were manually inoculated (Jones *et al*, 1977) with RMV (+V) whereas others were maintained without RMV infection (-V). The ramets were split repeatedly and maintained in a glasshouse, in pots (0.8 l capacity) containing a peat:sand mix, for about one year prior to the start of the experiment. For the experiment, 30 plants of each of the four treatment combinations (+E -V, -E-V, +E+V, -E+V) were placed on trolleys in a glasshouse. The position and orientation of the trolleys was changed weekly. The plants were harvested on six occasions over a 30-week period. At each harvest, all plants were cut at a height of 5 cm, the cut herbage was dried overnight at 85^{o}C and the dry weight for each plant was recorded. The herbage dry weight for each plant at each harvest was summed to obtain the accumulated yield for each plant over 5 harvests (data from the first harvest was not included because the cutting treatment was introduced at this time). For each E x V treatment combination, half of the plants were cut again to a height of 1 cm, but the cut material was not included in the dry weight. The plants received Miracle-Gro™ fertilizer (15%N:26%P:12%K + trace elements) in solution twice weekly, and each plant received 2 g of Chempack Summerlong™ slow-release fertilizer (40%N) after the second harvest and 10 g after the fourth harvest.

RESULTS

Data for the first harvest is not included in the results because the cutting height treatment was introduced at this time. The main effects of treatments were that the herbage dry matter yield accumulated over harvests 2-6 was 6.6% higher for +E plants than for -E ($P< 0.05$), 31.3% higher for -RMV plants than for +RMV ($P< 0.001$), and

106.2% higher for plants cut at 5 cm height than for those cut at 1 cm ($P< 0.001$). The results for the eight treatment combinations are shown in Figure 1. The accumulated yield for +E+V plants was 13.7% higher than -E+V ($P< 0.05$) at the 5 cm cut and 65.0% higher ($P< 0.05$) at the 1 cm cut; there was no significant effect of E with -V plants.

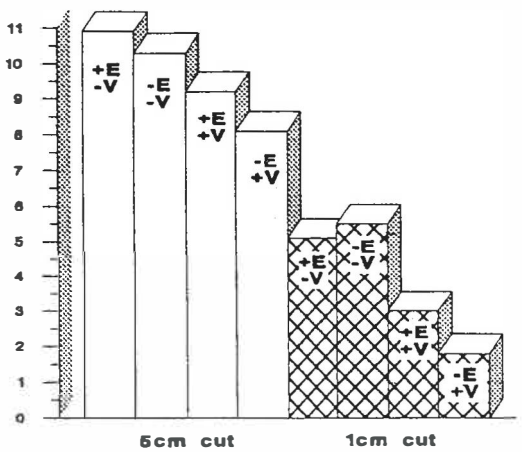


Figure 1 Accumulated herbage yield (g DM) from five harvests of *Lolium perenne* plants with and without infection by *Acremonium lolii* (±E) and ryegrass mosaic virus (±V) and cut at a height of 1 cm or 5 cm.

DISCUSSION

The results are in agreement with the previous finding (Lewis & Day, 1993) that the effect of virus infection in *L. perenne* plants is reduced when *A. lolii* infection is also present. Only RMV infection was included in the present work, whereas both RMV and barley yellow dwarf virus were present in the previous work. The results obtained for the two cutting heights differ from those reported by Wilkinson (1993), using field plots of *Festuca arundinacea* with and without infection by *A. coenophialum*. In the previous work, *Acremonium* infection increased herbage yield to a greater extent under a moderate

defoliation regime than under a severe one, whereas the reverse was recorded in the present work. The conclusion from the present work is that +E plants were more tolerant than -E of stress caused by virus infection and severe defoliation. The mechanisms involved in this stress tolerance are unknown.

REFERENCES

Jones, M.B.; Heard, A.J.; Woledge, J.; Leafe, E.L. 1977: The effect of ryegrass mosaic virus on carbon assimilation and growth of ryegrasses. *Annals of Applied Biology* **87**: 393-405.

Lewis, G.C.; Day, R.C. 1993: Growth of a perennial ryegrass genotype with and without infection by ryegrass endophyte and virus diseases. *Tests of Agrochemicals and Cultivars, 14 (Annals of Applied Biology, **122**, Supplement)*: 144-145.

Lewis, G.C.; Vaughan, Brenda 1995: Evaluation of hydroponic culture of ten genotypes of perennial ryegrass on infection by the fungal endophyte *Acremonium lolii*. *Tests of Agrochemicals and Cultivars, 16 (Annals of Applied Biology, **126**, Supplement)*: 80-81.

Wilkinson, S.R. 1993: Influence of endophytic infection of K31 tall fescue on yield response to irrigation, cutting management and competition with Tifton 44 bermudagrass. *Proceedings of the Second International Symposium on Acremonium/Grass Interactions, New Zealand, 1993*: 189-192.

PRACTICAL ENDOPHYTE-WORK AT THE BARENBRUG GRASS BREEDING DEPARTMENT

Wout Bouter & Gerard van 't Klooster

BARENBRUG RESEARCH, the NETHERLANDS

INTRODUCTION

The way a commercial grass-seed company does endophyte-research differs a lot from the fundamental research that is done by universities and government institutes. The reason for this difference is that laboratories at breeding companies only can do applied research on subjects that will possibly result in better (selling) varieties. The commercial motive is always present in the research at breeding companies.
We will give a rough presentation of the endophyte-work at the BARENBRUG grassbreeding department. Further we will mention some research-subjects that, to our opinion are useful to be examined by institutes and universities.

ENDOPHYTE-SCREENING IN SEEDS & LEAVES

More and more new turfgrass varieties are built on endophyte-free or endophyte-containing clones. For this reason a lot of screening has to be done in "wild-type" collections and other breeders material. Also in relation to the inoculation, the multiplication, the killing and the storage-work, many seeds and plants have to be screened for endophytes. For this screening work we use the wellknown leaf sheaths & seed staining techniques with aniline blue solution. For the future it will be necessary to have quick, practical and reliable methods for screening viable endophytes in seeds.

Infection of grasses with endophytes

We use for this "artificial infection" a modified version of the seedling inoculation technique developed by Latch & Christensen, New Zealand.

We can achieve the following (average) infection percentages:

For perennial ryegrass (Lolium perenne)	20%
For red fescue (Festuca rubra)	60%

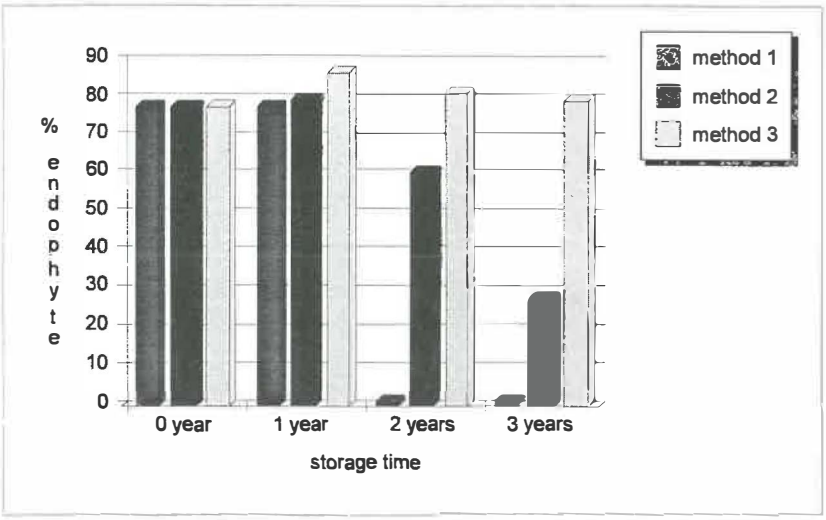
BARENBRUG uses this technique for infecting promising turfgrass varieties with endophytes, because of the enhanced insect-resistance and the enhanced stress tolerance. BARENBRUG already has commercially available several turftype perennial ryegrass varieties with high endophyte-levels. In 1998 market-introduction of 2 high endophyte red fescue varieties is planned.

Until now we (and everybody else) failed to get stable infection of Kentucky bluegrass (Poa pratensis) with non-choking endophytes.

VIABILITY OF THE ENDOPHYTE IN PERENNIAL RYEGRASS SEEDS DURING STORAGE

For a company like BARENBRUG, who trades grass seed this is important research. Although most seed has a rapid turnover, in practice some seed stocks (and basicseed stocks!) will remain in our warehouses for a number of years.

Three years ago we started a storage trial and the interim results are reported here. Seeds of perennial ryegrass, which had good germination and 11,5% seed moisture, were stored at three places.



method 1 uncontrolled, ambient, fluctuating conditions
method 2 seed storage warehouse (uncontrolled in temperature and RH)
method 3 controlled seed storage (12 dgr. C. and 30% RH)

These results agree with the data from literature. To maintain endophyte viability during storage of ryegrass low humidity/cool environment seed stores are needed.

KILLING ENDOPHYTES IN GRASS SEED

Work is being done to apply a reliable method of killing endophytes in ryegrass and tall fescue.
For killing endophytes in grass seed BARENBRUG has two important reasons:

1. The forage grasses should preferably be endophyte-free, because of the toxicities in animals caused by endophytes. So this method can be important for the production of endophyte-free foragegrass varieties.
2. By killing the endophytes in several genotypes of ryegrass and tall fescue we produced isogenetic material with and without endophytes for "ecological-fitness" trials in France and the United States of America.

By using a method, developed by Healther M. Nott we succeeded in killing most of the endophytes in seed lots of ryegrass and tall fescue. We still face two problems:

1. It seems impossible to kill the endophytes without strong decline in the germination of the seeds.
2. This method is only suitable for small seedlots, unfortunately not for big bags with grass seed.

CONCLUDING REMARKS

All the current endophyte-work at the BARENBRUG Grass Breeding Department is based on research formerly carried out by universities and institutes. Therefore the commercial grass seed companies need more fundamental research from the universities and institutes. In addition to the research-goals mentioned above, BARENBRUG would encourage the development of a real "Endosafe" (an endophyte which produces the desirable chemicals like peramine and which does not produce the undesirable chemicals that cause animal toxicities).

Development and sporulation of chosen fungi of *Drechslera* and *Bipolaris sp.* on several culture media.

AGNIESZKA PŁAŻEK, BOGUSŁAWA MUSIAŁ

Plant Breeding and Acclimatization Institute, Department of Fodder Crops, Kraków, POLAND.

ABSTRACT

The laboratory works related to resistance breeding are the aim of preparing the satisfactory amount of inoculum used in artificial infection in the greenhouse conditions. The choice of proper medium on which the pathogen would form a great amount of spores is very important. Some fungi can produce the pigments. In the case, when studied mycelium does not create any conidia, the ability to produce pigment can be useful by identification of given pathogen.

The purpose of this work was the examination of development of *Drechslera dictyoides, D. avenae, D. siccans* and *Bipolaris sorokiniana* and their ability to produce spores and pigments on seven various media. In the study the following media were used: agar, maltose, maize, potatoe (PDA), Czapek-Dox, Łacicowa and medium of autoclaved grass seeds.

The medium of autoclaved seeds was the best one for the sporulation of all examined pathogens. The ability to produce a pigment was observed in *D. dictyoides* (dark red, on the PDA and maltose medium) and in *D. siccans* (orange, on the PDA, maltose, Czapek-Dox and maize medium).

Keywords: *Bipolaris sp.,* culture media, *Drechslera sp.,* pigment, sporulation.

INTRODUCTION

The most common pathogens infecting grass leaves are fungi of *Drechslera sp.* They cause not only fresh mass decrease but also the deterioration of the fodder grass

quality due to toxins produced by some of these fungi (Davies and Williams 1970, Shotwell and Ellis 1976, Rodricks et al. 1977). Our long-term observation demonstrates that a few species of *Drechslera* fungi simultaneously appear on the fescue leaves. These are *D. dictyoides, D. avenae, D. siccans* and *Bipolaris sorokiniana* (syn. *Drechslera sorokiniana*).

The laboratory works related to resistance breeding are the aim of storing of the pathogen isolates without loss their virulence and they should prepare the satisfactory amount of inoculum used in artificial infection in the greenhouse conditions. The choice of proper medium on which the pathogen would produce a great amount of spores is very important.

Mycelium development and appearance of studied pathogens is depended on kind of culture medium and laboratory conditions - temperature and light. The ability of some fungi to produce the pigments can be useful by identification of given pathogen, specialy, when mycelium does not form any conidia.

The purpose of presented work was the evaluation of the development of the above mentioned fungi mycelia and their ability to produce spores and pigment on seven various media.

MATERIAL AND METHOD

In the study there were used the following media: agar, maltose, maize, potatoe (PDA - potatoe dextrose agar), Czapek-Dox, Łacicowa and medium of autoclaved seeds. The Łacicowa medium was prepared specialy for development of *Drechslera* fungi (Łacicowa 1970). The medium of autoclaved seeds was prepared of meadow fescue caryopses put directly into the plastic dishes, covered with a litle amount of destilated water and autoclaved for 10 minutes in 121° C. The other media were poured into plastic-dishes. Next all media were inoculated with the small pieces of mycelia of all examined pathogens. All isolates used in the study were obtained in our institute. After 8-day-incubation in darkness and temperature of 20° C the differences between development and appearance of mycelia and pigment producing were noticed. Next the dishes were lit with NUV light in order to stimulate sporulation. After seven days the ability to form spores related to media was estimated by means of light microscope.

Table 1.

The appearance, sporulation and pigment producing of *Drechslera sp.* and *Bipolaris sorokiniana* mycelium on several media.

Media	D.dictyoides	D.avenae	D.siccans	B.sorokiniana
PDA	White, fluffy mycelium, dark red pigment	white-grey, fluffy mycelium, a lot of spores	grey mycel. orange pigment, a lot of spores	black mycelium, a lot of spores
maltose	white-grey, a little fluffy mycelium, dark red pigment	white-grey, fluffy mycelium, several spores	grey mycelium with grey pubes-cence, orange pigment	white-grey, fluffy mycelium, several spores
agar	pellucid, branched hyphae, several spores	pellucid, branched hyphae, several spores	pellucid hyphae with single-spores	pellucid, branched hyphae with single-spores
maize	grey-green, fluffy mycelium, scant spores	grey-white-green, fluffy mycelium, a lot of spores	grey mycelium with white pube-, scence, orange pigment, a lot of spores	white, fluffy mycelium
Łacicowa	white-rose, cloddy mycelium	white-grey, fluffy mycelium	white, cloddy mycelium	white-rose cloddy mycelium
Czapek-Dox	white, delicate fluffy mycelium	white-grey, delicate fluffy mycelium, several spores	grey mycelium, a little orange pigment, numerous spores	white-grey, fluffy mycelium, several spores
autoclaved seeds	white mycelium, a lot of spores	white-grey mycelium, a lot of spores	grey mycelium, plenty of spores	grey mycelium, plenty of spores

RESULTS

The table 1. demonstrates the appearance of all studied species of *Drechslera* and *Bipolaris sorokiniana* on several media. The ability to produce pigment was observed only in *Drechslera dictyoides* (dark red on the PDA and maltose medium) and *D. siccans* (orange on the PDA, maltose, Czapek-Dox and maize medium).

The greatest variety in the appearance of mycelium related to medium content was observed in *Bipolaris sorokiniana*. *D. avenae* formed the same kind of mycelium on all media without agar medium. On this medium all studied species of fungi produced delicate hyphe with a few conidial stalks with spores. The greatest problem with spore formation was observed by *D. dictyoides*. The best medium for the sporulation of all examined pathogens was the medium of autoclaved seeds.

DISCUSSION

The descriptions of appearance of *Drechslera sp.* mycelia on different media are not often published. Our observations demonstrate that look of these mycelia can change in dependenance of temperature, light and kind of artificial medium. In laboratory conditions conidia can show abnormal shapes and dimensions. In this case and when mycelium does not form conidia the ability to produce of pigments can be very useful by identification of given pathogen (Scott 1995). The works characterizing the appearance, sporulation and pigment producing of pathogens are very valuable for studies in laboratory.

REFERENCES

Davies, H. Williams, A.E., 1970, The effect of mildew and leaf bloth on yield and quality of cv. Lior Italian Ryegrass. Plant Pathology 19: 135-138.

Łacicowa, B., 1970, Badania szczepów *Helminthosporium sorokinianum* (=*H. sativum*) oraz odporności odmian jęczmienia jarego na ten czynnik chorobotwórczy. Acta Mycologica 6(2): 187-248.

Rodricks, J.V., Hesseltine, C.W., Mehlman, M.A., 1977, Mycotoxins in human and animal health. Pathotox Publishers, Inc., Park Forest South. Illinois.

Scott, D.B., 1995, *Helminthosporia* that cause leaf spots on small-grain cereales in South Africa, 107-137, In: *Helminthosporia* metabolites, biology plant diseases *Bipolaris, Drechslera, Exserohilum.* Editor J. Chelkowski, Institute of Plant Genetics Polish Academy of Sciences.

Shotwell, O.L., Ellis, J.J., 1976, *Helminthosporium, Drechslera* and *Bipolaris* toxin, 318-343, In: Mycotoxins and other fungal related food problems, J.V. Rodricks (red), Advances in chemistry, Ser. 149, Am. Chem. Soc., Washington D.C.

The microflora of short rotation ryegrass and westerwolths ryegrass fertilised with cattle slurry. Part II. Fungi.

Sadowski Cz.[1] , Kluczek J. P.[2] , Skinder Z.[3]

[1] Dep. of Phytopathology, [2] Dep. of Animal Higiene, [3] Dep. Cultivation of Soil and Plants.
University of Technology and Agriculture, Kordeckiego 20, 85-225 Bydgoszcz, Poland.

ABSTRACT

Mineral and slurry fertilisation were applied to pure stands of short rotation westerwolths ryegrasses and their mixtures with persian clover. The fungi of slurry, soil, roots, root nodules, fresh and dry crop, and plant yields were estimated. In the plant material derived from the plots fertilised with slurry the number of fungi was considerably higher and the range of species greater. Applying higher doses of slurry was correlated with an increase of fungi in soil, on roots and the aboveground parts of plants. Moreover, the fertilisation with slurry involved the risk of contamination with pathogenical fungi. Drying of plant material greatly reduced the incidence of mycoflora. Reduced numbers of fungal colonies were isolated from ryegrasses cultivated in a mixture with persian clover compared to ryegrass pure stands.
Keywords: *clover, contamination, fertilisation, fungi, mycoflora, pathogenic, ryegrass, slurry.*

INTRODUCTION

Slurry is regarded as a valuable manure. However, when used without proper preparation, it can cause pollution of soil, air, surface and ground water. Applications of slurry in untested ways or using slurry in its crude form may affect the biological balance of microflora in these areas. Some slurry microorganisms and the toxic substances excreted by them can have a harmful affect on the environment and may lead to

environmental degradation (Kluczek 1986, Kluczek at al. 1994, Sadowski at al. 1994 a, 1994 b).

The fungi which can be the cause of plant diseases and, indirectly, can affect people and animals may be introduced through fertilising with cattle slurry. The metabolites causing mycotoxycoses are especially pathogenic, and refer mainly to the genera Aspergillus, Penicillium and Fusarium (Aleksandrowicz and Smyk 1971, Kothary et al. 1984). The aim of this investigation was to compare cattle slurry and mineral fertilising methods.

METHODS

Short rotation and westerwolths ryegrasses in pure stands and in mixtures with persian clover were fertilised with cattle slurry and with mineral fertilisers. The experiment was carried out with the randomised sublocks method, two-factorial, in four replications. One plot was of 20 m^2. The mineral fertiliser doses per ha were: P_2O_5 - 120 kg , K_2O - 2 x 80 kg , N - for the grasses in pure stand 3 x 80 kg , and for the mixtures 3 x 30 kg. Cattle slurry doses corresponded with mineral fertilisers and were; 3 x 54,2 m^3/ha for grasses snd 3x 20,5 m^3/ ha for mixtures.

Cattle slurry, soil from under the plants fertilised with slurry, green crop and dry matter were examined mycologically. Methods were presented in part I this paper. The green crop yield was estimated after each cut and total protein content and crude fibre content were determined.

RESULTS

Mycological analysis showed a large number of fungi in the examined slurry samples. The number of fungi found in 1g of cattle slurry was from 2,77 x 10^5 to 9,57 x 10^6 (Table 1). The genera *Aspergillus clavatus, Candida albicans, Candida krusei* and *Sacharomyces cerevisiae* were isolated from each sample.

Table 1. The fungi of investigated cattle slurry

Aspergillus clavatus, A. repens, A. spp., Candida aerogenes, C. albicans, C. guilliermondii, C. krusei, C.mesentrica, C. parakrusei, C. pseudotropicalis, C. stelloidea, C. tropicalis, Cryptococcus neoformans, C. spp., Fusarium graminearum, F. spp., Geotrichum candidum, Mucor mucedo, M. spp., Penicillium notatum, P. viridicatum, P. spp., Rhizopus spp., Saccharomyces cerevisiae, Torulopsis glabrata, Trichoderma lignorum, T. spp.
Colony count in 1 g 2,77 x 10^5 -9,57 x 10^6

From the soil treated with slurry, after the first dose, from 2,12 x 10^4 to 4,22 x 10^5 fungi were isolated from 1g of dry soil while from the soil, treated with mineral fertilisers from 3,12 x 10^2 to 4,21 x 10^4. After the second slurry dose from 3,69 x 10^5 to 4,51 x 10^8 fungi were isolated while at the same time from 1g of dry soil treated with mineral fertilisers from 2,59 x 10^3 to 4,23 x 10^5 fungi were isolated. The was no significant difference in the number of fungi isolated on agar medium with blood, on Sabouraud and Czapek medium.

Mycological analysis of the plants showed that fertilising slurry significantly increased the amount of fungi. From 1g of plant roots 3,13 x 10^4 to 2,79 x 10^4 fungi were isolated. The number of fungi isolated from the roots of plants fertilised with mineral fertilisers was significantly lower - 2,87 x 10^2 to 2,93 x 10^3 (Table 2).

Mycological analysis of root nodules also showed that the number of fungi in the nodules from the plots fertilised with slurry was considerably higher - 2,86 x 10^3 to 3,31 x 10^4 than in the nodules from the plots treated with mineral fertilisers - 31,3 to 40,2. The comparison made between the isolated fungi shows clearly that slurry fertilisation affected the nodule mycoflora (Table 3).

Table 2. The fungi of roots of plants fertilised with cattle slurry and mineral fertilisers

Cattle slurry	Mineral fertilisers
Aspergillus clavatus, A. repens, A. spp., Candida aerogenes, *C. albicans, C. guilliermondii, C. krusei, C. lipolytica, C. mesenterica, C. parakrusei, C. pseudotropicalis, C. stelloidea, C. tropicalis, Cryptococcus neoformans, Fusarium graminearum, F. spp., Geotrichum candidum, Mucor mucedo, M. spp., Penicillium notatum, P. viridicatum, Rhizopus spp., Saccharomyces cerevisiae, Torulopsis glabrata, T. spp., Trichoderma lignorum*	*AsAspergillus spp., Candida albicans, C. krusei, C.parakrusei, C.pseudotropicalis, C. stelloidea, C. tropicalis, Mucor mucedo, M. spp., Penicillium spp., Rhizopus spp., Saccharomyces cerevisiae*
Colony count in 1g	
$3{,}13 \times 10^4$ -$2{,}79 \times 10^6$	$2{,}87 \times 10^2$ - $2{,}93 \times 10^3$

Table 3. The fungi of root nodules of plants fertilised with cattle slurry and mineral fertilisers

Cattle slurry	Mineral fertilisers
Aspergillus clavatus, A. repens, A. spp., Candida aerogenes, *C. albicans, C. guilliermondii, C. krusei, C. lipolytica, C. mesenterica, C. parakrusei, C. pseudotropicalis, C. stelloidea, C. tropicalis, Cryptococcus neoformans, Fusarium graminearum, F. spp., Geotrichum candidum, Mucor mucedo, M. spp., Penicillium notatum, P. viridicatum, P. spp., Rhizopus spp., Saccharomyces cerevisiae, Torulopsis glabrata, T. spp., Trichoderma lignorum*	*Aspergillus spp., Candida krusei, C. lipolytica, C. stelloidea, C. tropicalis, Mucor mucedo, M. spp., Penicillium spp., Rhizopus spp., Saccharomyces cerevisiae*
Colony count in 1g	
$2{,}86 \times 10^3$ - $3{,}31 \times 10^4$	$3{,}13 \times 10^1$ - $4{,}02 \times 10^1$

Slurry fertilisation considerably increased both the number of genera and the number of species on the aerial parts of plants. In 1g of leaf and stem mass derived from the plots fertilised with slurry 3,62 x 10^2 to 5,46 x 10^6 fungi were found while in the plant material from the minerally treated polts - 3,37 x 10^2 to 5,06 x 10^3. Moreover, it was observed that applying higher doses of slurry was correlated with the increase of the total fungi amount on slurry - treated plots (Table 4).

Table 4. The fungi of leaves and stems of plants fertilised with cattle slurry and mineral fertilisers

Cattle slurry	Mineral fertilisers
Aspergillus clavatus, A. repens, A. spp., Candida albicans, C. guilliermondii, C. krusei, C. lipolytica, C. mesenterica, C. parakrusei, C. pseudotropicalis, C. stelloidea, C. tropicalis, Cryptococcus neoformans, Fusarium graminearum, F. spp., Geotrichum candidum, Mucor mucedo, M. spp., Penicillium notatum, P. viridicatum, P. spp., Rhizopus spp., Saccharomyces cerevisiae	*Aspergillus spp., Candida albicans, C. krusei, C. parakrusei, C. pseudotropicalis, C. stelloidea, C. tropicalis, Geotrichum candidum, Mucor mucedo, M. spp., Penicillium spp., Rhizopus spp., Saccharomyces cerevisiae*
Colony count in 1g	
3,62 x 10^2 - 5,46 x 10^6	3,37 x 10^2 - 5,06 x 10^3

Hay obtained by natural drying of plants was also analysed. The drying process significantly decreased the amount of fungi. However, the number was higher in hay obtained from slurry fertilised plants than in hay derived from mineral treated plants, 2,25 x 10^3 to 3,65 x 10^4 in 1g of dry matter for the former and only 20,2 to 278,0 for the latter (Table 5).

Hay yields from the slurry treated plots were similar to the hay yields obtained from the plots fertilised with the equivalent nitrogen dose. The slurry fertilisation did not have any affect on the total protein and crude fiber content compared to the mineral fertilisation of plants.

Table 5. The fungi of dried forage fertilised with cattle slurry and mineral fertilisers

Cattle slurry	Mineral fertilisers
Aspergillus clavatus, A. repens, A. spp., Candida albicans, C. guilliermondii, C. krusei, C. lipolytica, C. mesenterica, C. parakrusei, C. pseudotropicalis, C. stelloidea, C. tropicalis, Cryptococcus neoformans, Fusarium graminearum, F. spp., Geotrichum candidum, Mucor mucedo, M. spp., Penicillium notatum, P. viridicatum, P. spp., Saccharomyces cerevisiae, Torulopsis glabrata, Trichoderma lignorum	*Aspergillus spp., Candida krusei, C. mesenterica, C. pseudotropicalis, C. stelloidea, C. tropicalis, Mucor mucedo, M. spp., Penicillium spp., Saccharomyces cerevisiae*
Colony count 1g	
$2{,}35 \times 10^3$ - $3{,}65 \times 10^4$	$2{,}02 \times 10^1$ - $2{,}85 \times 10^2$

DISCUSSION

The investigation showed that there was an undesirable affect of cattle slurry fertilisation on the mycoflora of soil and plants. Fertilising with slurry containing large quantities of fungi resulted in the number of fungi increasing in both soil and on plants. This factor can be very harmful because of the changes in the composition of soil mycoflora which has a steady biological balance. Cattle slurry fertilisation increased the amount of pathogenic soil fungi. The undersirable influence of slurry fertilisation on soil mycoflora and on biological balance of soil biocenosis was earlier shown by Ajello (1981), Boutin and Moline (1987), Kothary et al. (1984). The changes in composition of soil mycoflora caused by slurry fertilisation can increase the danger associated with mycotoxins. Investigations carried out by various authors show that typical soil microorganisms within a settled balance have the properties of degradation or adsorption of mycotoxins. Destroying this balance can cause disturbances in the functioning of such microorganism defence systems. Pathogenic microorganisms and their toxins found in cultivated fodder plants can have a harmful effect on the economics of animal production.

In large numbers they can even cause animal death (Duke 1985, Rylander and Snella 1983, Strauch and Bertoldi 1986).

Green crops containing fungi, and subsequently dried material can be a source of fungal infection in animals. This is an important practical problem since dissemination of contagions diseases can occur. Pathogenic fungi found in dried crops result in the nonutilisation of the material as fodder. Although there were slight differences in the composition of fungal genera in the various plant combinations, *Candida, Geotrichum, Cryptococcus, Torulopsis, Aspergillus, Penicillium and Fusarium* were most frequently found in dry matter derived from slurry fertilised fields. However, the proportions of obtained yields showed that cattle slurry and mineral fertilisers were equally useful in the cultivation of the investigated grasses and mixtures with persian clover. The slurry fertilisation did not have any effect on the total protein and crude fibre content compared to the mineral fertilisation of plants.

On the basis of these results, some soil and plant contamination with mycoflora as well as disturbances of biological balance are evident. Protection of fodder quality results in the protection of both economic breeding effects and animal and human health.

REFERENCES

Ajello, L. 1981: Natural habitats of the fungi that cause pulmonary mycoses. pp. 31-41. Preusser (*eds*) Medical Mycology. Zentralblatt fur Bakteriologie und Hygiene. Suppl. & Gustav Fischer Verlag, Stuttgart-New York.

Aleksandrowicz, J.; Smyk, B. 1971: Mycotoxins and their role in ethiology of tumour diseases of humans ans animals. (in Polish). *Polish Archives of Internal Medicine.* 47: 331-339.

Boutin, P.; Moline, J. 1987: Health and safety aspects of compost preparation and use. pp. 198-206 *in*: Compost; Production quality and use (eds. M. de Bertoldi et al.). Elsevier Applied Science, London-New York.

Duke, T. W. 1985: Uncertaintly and environmental risk assessment. *Environmental Science Technology* 19: 99-105.

Kluczek, J. P. 1986: Sanitary and hygienic aspects of the animals sevege problem. (in Polish) BTN. *Prace Komisji Nauk Rolniczych i Biologicznych* 24: 43-88.

Kluczek,J.P.; Skinder,Z.; Sadowski,Cz. 1994: The microflora of short totation ryegrass and westerwolths ryegrass fertilised with cattle slury, Part I. Bacterial flora, IOBC/WPRS Bulletin.Vol.17 (1): 147-153.

Kothary, M. H.; Chase, T.; McMilan, J. D. 1984: Levels of Aspergillus fumigatus in air and in compost at sewage sludge compositing site. *Environmental Pollution. (Ser. A),* 34:1-9.

Rylander, R.; Snella M. C. 1983: Endotoxins and the lungcellular reactions and risk for disease. *Progress in Allergy.* 33: 332- 339.

Sadowski, Cz.; Skinder, Z.; Kluczek, J.P. 1994a: The effect of cattle slurry fertilization of Westerwolths ryegrass cultivated in pure stand and in mixture with Persian clover on the yield and mycoflora composition. (in Polish). *Genet. Pol.* 35A: 321-326.

Sadowski, Cz.; Skinder, Z.; Kluczek, J.P. 1994b: The effect of fertilization of persian clover (*Trifolium resupinatum* L.) with cattle slurry on the composition of microorganisms. (in Polish). *Zesz. Probl. Post. Nauk Rol.* Z. 414: 289-298.

Strauch, D.; de Bertoldi, M. 1986: Microbiological specification of disinfected sewage sludge. pp. 178-186 *in*: Processing and use of organic sludge and liquid agricultural wastes, L'Hermite, P. *ed.* Dordecht / Boston / Lancaster / Tokyo, D. Reidel Publ. Company.

Lolium latent virus - a frequent pathogen in breeding stations

Winfried Huth

Federal Biological Research Center, Institute for Biochemistry and Plant Virology
Messeweg 11/12, D-38104 Braunschweig Germany

ABSTRACT

The prevalence and some properties of a virus called Lolium latent virus (LLV) are discribed. The virus was found in plants of *Lolium* spp. from breeding stations only. Most of these plants were infected by a mixture of LLV and ryegrass mosaic virus.

Keywords : Lolium latent virus, ryegrass mosaic virus, *Lolium* spp., distribution, symptoms

Several viruses are frequent pathogens of wild grasses in patures and in borderies around arable fields as well as of cultivated grasses in breeding stations. Although virus infections reduce growth and yield of plants some virus diseases are unnoticed because the mostly tend to be mild symptoms. Ryegrass mosaic virus (RMV) is one of these viruses which is common at least in Europe. During a study of the prevalence and distribution of this virus in breeding stations in plants infected by RMV, a second virus was detected. When mechanically transmitted to *Lolium perenne* and *L. multiflorum* all infected plants remained symptomless infected. Therefore and because no relationship to any of the known gramineacous viruses were found this virus was called Lolium latent virus (LLV). The particle structure of the virus resembles that of potexviruses although it differs in particles length, molecular weight of the coat protein and nucleic acid size (HUTH et al., 1995). Hitherto only one potexvirus, foxtail mosaic virus (FMV, PAULSEN and NIBLETT, 1977) was known to infect *Gramineae*. LLV is serologically not related to FMV.

SYMPTOMS ON INFECTED PLANTS

Whereas *Lolium* spp. remained symptomless, disease expression in plants infected by both LLV and RMV was much stronger than with RMV alone. Instead of a mostly mild light green mosaic caused by RMV, the leaves of plants infected by both RMV and LLV showed a severe mosaic with yellow to white spots. In greenhouse trials plants infected with both LLV and RMV remained smaller and their oldest leaves and finally the whole plants died earlier than counterparts containing either pathogen alone. Although incomplete information is available, it is probably that naturally infected plants are similarly stronger damaged and that their growth and yield when infected by both viruses will be reduced to a greater extend than when infected with RMV alone.

HOST RANGE OF LLV

The range of hosts is one of the properties of viruses. In addition to *Lolium* spp., some further grass species (*Avena sativa, A. byzanthina, Bromus tectorum, B, sterilis, Briza maxima, Dyctylis glomerata, Hordeum vulgare, Lagurus ovatus, Secale cereale*) are susceptible to LLV when inoculated with virus-containing plant sap. Most of these grass species are much more susceptible to LLV than *Lolium* spp.. Whereas only a few plants of *L. perenne* became infected after virus inoculation, all plants of *Bromus sterilis*, *B. tectorum* and *Briza maxima* were infected. Two weeks after inoculation, short pale green streaks appeared on the later developed leaves. Although only plants of *Lolium* spp. were found naturally infected I cannot exclude the possibillity that some other grass species are infected naturally.

DISTRIBUTION

LLV was found in 4 of 5 breeding stations in Germany and at a further one in The Netherlands (Table 1, NL). From a total of 260 virus containing-plants of ryegrass 86 (34%) were infected by RMV, 20 (8%) plants contained only LLV. The largest number of 145 (58%) plants was infected by a mixture of RMV and LLV. LLV was not found in a breeding station near Malchow close the Baltic Sea or in fields of the Federal Office of

Plant Varieties (Bundessortenamt) at Scharnhorst (Lower Saxony). Furthermore during a survey of plants showing RMV-like symptoms outside breeding stations, no plants were found to be infected by LLV.

Table 1: Ratio of plants of *Lolium perenne*[1] and *L. multiflorum*[2] from several breeding stations infected by ryegrass mosaic virus (RMV), Lolium latent virus (LLV) or a mixture of both viruses

Breeding station near	No. inf. plants	infected by		
		RMV	LLV	RMV+ LLV
Asendorf[1]	63	24	12	27
Freising[1]	65	29	1	26
Hohenlieth[1]	88	15	4	69
Lelysad[2] (NL)	39	14	3	22
Steinach[1]	5	4	0	1
Sum	260	86 (34%)	20 (8%)	145 (58%)

TRANSMISSION

In nature, viruses are specifically transmitted by special vectors such as insects, mites, nematodes or fungi. Therefore, the spread of viruses as well as their economical and agronomical importance mostly depends on the occurrence of their vectors. Not all vectors have been tested but the following cereal aphids (*Methopolophium dirhodum*, *Myzus persicae*, *Rhopalosiphum maidis*, *Rh. padi*, *Sitobium avenae*) were unable to transmitting LLV. In only a very few instances (8/483) *R. padi* seemed to be an inefficient vector of LLV.

LLV as the only pathogen in *Lolium* spp. does not reduce growth and yield of infected plants. It enhanced the symptom expression caused at least by RMV with which it occurs mixedly infected in plants. Because LLV were not found in wild grasses from

the great number of infected plants in breeding stations is followed, LLV there may be spread by cloning of infected plants; it was not found in scattered wild individuals. However, the absence of LLV may be due in part to the synergy with RMV

REFERENCES

HUTH; W., D.-E. LESEMANN, H.-J. VETTEN, R. GÖTZ, 1994: Ein neues Gramineenvirus aus*Lolium* spp.. Mitteil. Biologische Bundesanstalt für Land- und Forstwirtschaft, Heft 301, 217.

HUTH, W, D.-E. LESEMANN, H.-J. VETTEN, R. GÖTZ, 1995: Lolium latent virus - a possible new potexvirus isolated from *Lolium* spp.. Proc. Fourth Intern. Conf. Plant Diseases. 6 - 8 Decembre 1994, Bordeaux, 1095 - 1099.

PAULSEN, A. Q., C. L. NIBLETT, 1977: Purification and properties of Foxtail mosaic virus. Phytopathology 67, 1346 - 1351.

Secondary Metabolites

Secondary Metabolites from Endophytic Fungi

Karsten Krohn

Fachbereich Chemie und Chemietechnik, der Univ. GH Paderborn
Warburger Str. 100, D-33098 Paderborn

The role of secondary metabolites in plants, bacteria and fungi is not yet fully understood. However, it is assumed that these products may play an important role in chemical defence and communication. Although relatively few fundamental biosynthetic pathways exist, these result in large groups of natural products such as polyketides, amino acids, sugars, terpenes, and steroids - a fascinating variety of structurally different metabolites. This immense variety is demonstrated with a number of selected natural products isolated from endophytic fungi by our research group in cooperation with the microbiologists in Braunschweig. The structural diversity is the result of two phenomena: mixing of different biosynthetic pathways and chemical modification of the fundamental structures. Our poster shows an example of this latter chemical modification of one basic structure in the group of palmarumycins.

Thus, evolution has lead to a large number of antibiotic compounds (more than 10.000 different structures are known) with a large variety of different modes of action. Many of these compounds are simply „cytotoxic“ (in particular if they act on DNA) and are of little use as antibiotics for bacterial infections in humans. However, new lead structures for further chemical optimization are urgently needed for crop protection and medicinal chemistry. Thus, natural product chemistry plays an important role in this task, both now and in the future.

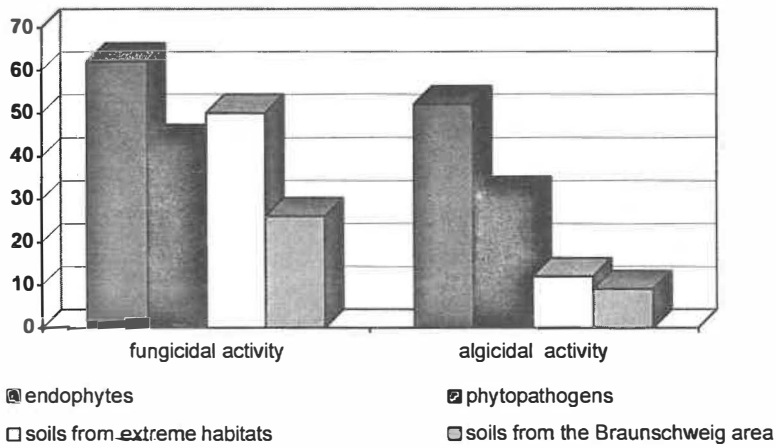
This lecture introduces the section on secondary metabloites from endophytic fungi.

A few general remarks shall demostrate the principles of

- strain selection
- screening for biological activity
- TLC investigations ("chemical and biological screening");
- sample purification;
- chemical structure elucidation.

Several years ago, we started a very productive cooperation with a group of microbioligists at the Technical University of Braunschweig (Prof. H. J. Aust; Dr. B. Schulz, and Dr. S. Draeger and coworkers). Initially, a great variety of fungi from different sources was tested in our the search for biologically active compounds. In the early stage of our investigations a large number of known compounds, in addition to the unknown metabolites, was isolated in the screening of microorganisms. It was thus very important to optimize the chances for finding new and biologically active natural products by careful selection of the organisms studied. After screening a large number of strains for fungicidal and algicidal acitvity, a statistical evaluation of the „hit rate" of fungi from the Braunschweig area, from extreme habitats (e.g. deserts, arctic areas, regions contaminated with heavy metals), from phytopathogenic fungi, and endophytic fungi was undertaken. Figure 1 shows the results, clearly demonstrating the high rate of fungicidal and algicidal (herbicidal) activities in endophytic fungi. Therefore, we have focused our interest on endophytic fungi.

Figure 1: Fungicidal and Algicidal Activity found in Fungi from Different Sources



Our colleagues in Braunschweig carefully selected the fungi which were later investigated for the isolation of biologically active secondary metabolites at our Institute in Paderborn. Every culture was tested for biological activity with a spectrum of different organisms that represent Gram-positve and Gram-negative bacteria, fungi, but also algae and seedlings: B.m. = *Bacillus megaterium*, E.c. = *Escherichia coli*, U.v. = *Ustilago*

violacea, M.m. = *Mycotypha microspora*, E.r. = *Eurotium repens*, F.o. = *Fusarium oxysporum*, C.f. = *Chlorella fusca, Lepidums sativum* (cress).

At this stage it was sufficient to use conventional agar plates and measure the inhibition zones to have a rough idea of the specific activity. One additional problem should be mentioned that is connected with the cultivation of endophytic fungi. Very often they produce more secondary metabolites on a semi-solid media than they do in submerged culture. Fernbach vessels were used for some time for cultivation, but they have recently been replaced by penicillin vessels which have a larger surface area. In addition, it is unfortunately sometimes very difficult to use fermenters for the cultivation because the hyphae congest the apparatus.

Furthermore, the extraction of compounds from semisolid aga is much more tedious than from a culture broth. Usually, a Waring blender is used to homogenate the culture before extraction with organic solvents such as ethyl acetate.

Having obtained the extract, the next task is to identify the biologically active compounds. Several techniques are used to obtain these "bioautograms". The oldest technique makes an imprint of the TLC plates on agar plates on which a test organism is grown. The compounds diffuse from the TLC plates into the agar and and show inhibition zones at the specific locations were active compounds were located. A disadvantage of this method is its dependency on the water solubility of the substances and the difficulty in exactly locating the zones of inhibition on the TLC. This method is still used for testing antibacterial activity. In testing for antifungal activity our collegues in Braunschweig use a different technique in which they spray the TLC with a suspension of *Cladosporium*. Activity is then visible as a white zone of inhibition on a green background, precisely locating the fungicidal compounds. A similar method was developed for herbicidal activity using the alga, *Chorella fusca*. Other tests for herbicidal or algicidal activity are more time consuming and do not precisely locate the active substances. Additionally, they are dependent on the water solubility of the substance and require larger amounts of the compound.

At this stage it should be mentioned that we also use "chemical screening". This is simply the application of a variety of different spray (spot tests) in order to visualize compounds of diverse chemical classes on TLC plates. The different spraying reagents give preliminary hints as to the chemical nature of the constituents produced in a fungal culture. The compounds that initially show no activity in any of the preliminary tests are subjected to a number of enzymatic tests by our industrial cooperation partners. These enzymatic tests are increasingly being developed to replace the traditional *in vivo* tests. Extensive automation allows for the testing of a very large number of compounds. The tests are being developed for pharmaceutical, fungicidal, herbicidal, and insecticidal targets.

Having located definite spots on the TLC by chemical or biological screening the next task is to isolate and purify the individual compounds. For this purpose the entire arsenal of chromatographic techniques is applied: column, layer, and HPLC chromatography using differnt adsorbents such as silica gel, reversed phase silica gel, and less frequently alumina and sephadex. *A very useful* technique is the separation on rapidly rotating silica coated discs (chromatotron). Repeated TLC is very often used in the final stage of the purification steps.

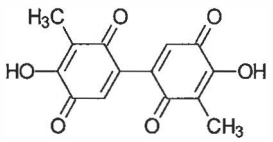
An important task is accomplished when the compound has been isolated in pure form from the crude extract. However, very often the most time consuming work is structure elucidation using the entire spectrum of spectroscopic measurements such as infrared (IR), ultraviolett/visible (UV/VIS), mass (MS), and - most importantly - nuclear magnetic resonance (NMR) spectroscopy. A lot of experience and training is required in the professional use of these techniques. Spectroscopic data bases such as "Specinfo" are used in our laboratory to assist the correct assignment of the spectroscopic data. Known compounds are identified by comparison with literature data. In this respect data bases of natural products such as the Chapman and Hall "Dictionary of Natural products on CD ROM" or *"Anti*base" from VCH publisher (author Prof. H. Laatsch, Göttingen) are extremely helpful.

In favorable cases crystals of good quality can be used for X-ray analysis of the samples and we are happy to have good instrumental facilities and cooperation partners both in Braunschweig (Prof. P. Jones) and Paderborn (Dr. U. Flörke). The absolute configuration can be illucidated only if the compound contains heavy atoms such as halogens.

Otherwise circular dichroism (CD) or optical rotation dispersion (ORD) can be used to get information about the absolute configuration. In this respect we also cooperate with other groups (Prof. Bringmann, Würzburg and Prof. S. Antus, Debrecen).

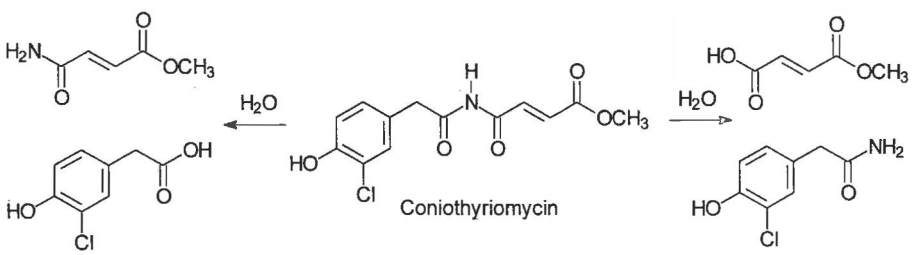
In the final section of this introduction I would like to present a few selected structures demonstrating the structural variety of the secondary metabolites. Interestingly, as quinone chemists, one of the first products isolated was a dimeric benzoquinone.

Figure 2. Structure of Dimeric Benzoquinone



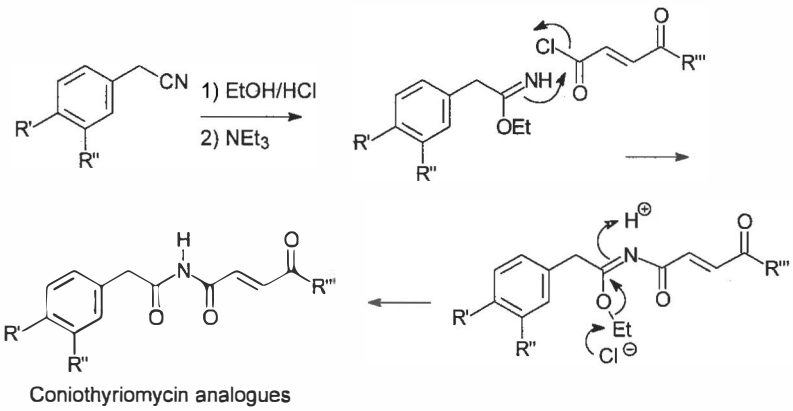
Another compound that almost became a developmental product, was an open chain imid called coniothyriomycin. It had been isolated from *Coniothyrium* sp. The metabolite showed excellent activity against a variety of fungi including *Botrytis cinerea*, a problematic pest in vineyards[1]. However, the antifungal activity was only effective for a short time: the compound decomposed into phenylacetic acid and fumaric acid derivatives upon hydrolysis with water (Fig. 3).

Figure 3. Structure of Coniothyriomycin and Decomposition with Water



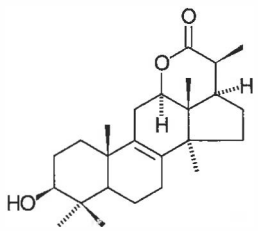
The relatively simple structure was also prepared by total synthesis yielding many derivatives. Unfortunately there was none that showed improved activity.

Figure 4. Total Synthesis of Coniothyriomycin and Derivatives



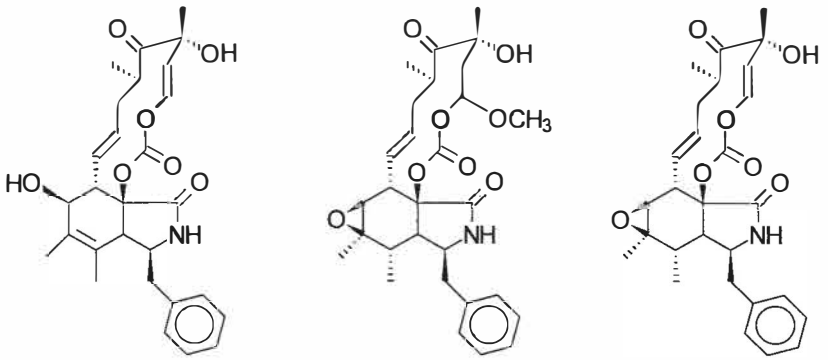
The next example shows the structure of a herbicidally active diterpene related to lanosterins from *Sporormiella australis*[2]. The growth of garden cress (*Lepidium sativum*) was inhibited, the seedlings showing spiral root growth.

Figure 5. Structure of a Lanosterin Lactone from *Sporormiella australis*



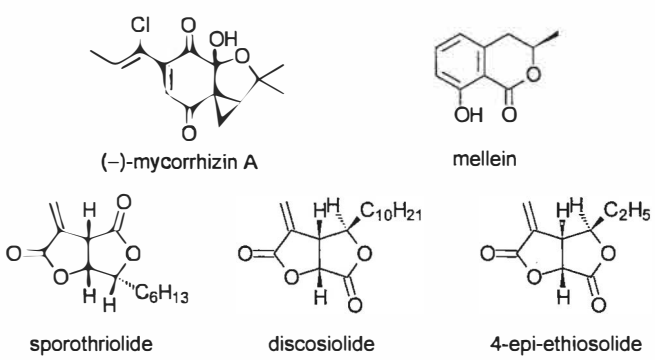
A totally different class of compounds are the well known cytochalsins isolated by our group from the endophytic fungus *Physalospora vacinii*[3]. The cytochalasins are a good example for the mixture of biosynthetetic pathways (in this case amino acids and ketides). This is one way in which nature creates the incredible diversity of natural products. A few examples of this group are shown in Fig. 6.

Figure 6. Structures of Cytochalasins Isolated from *Physalospora vacinii*



The diversity can also be very striking within one biosynthetic pathway. An example are a few compounds of ketide origin isolated from two strains of *Pezicula livida* shown in Figure 7. The melleins belong to the isocoumarins and occur very frequently[4,5]. New furofurandiones, sporothriolide, discosiolide, and 4-epi-ethiosolide from *Sporothrix* sp., *Discosia* sp. and *Pezicula livida*[6] are very poisonous due to the highly electrophilic exocyclic α,β-unsaturated lactone group (Michael acceptor).

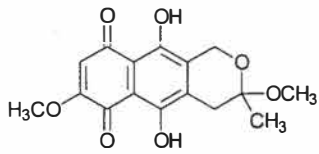
Figure 7. Compounds from *Pezicula livida, Sporothrix* sp., and *Discosia* sp.



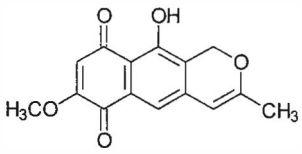
On the other hand, very simliar metabolites may also be isolated form one culture broth. In these cases a basic structure is modified by simple chemical modifications such as

reduction, oxidation, elimination, halogenation. One example is the tricyclic quinones isolated from *Fusarium* sp. (Fig. 8)[7].

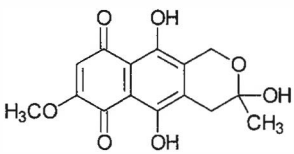
Figure 8. Products from *Fusarium* sp.



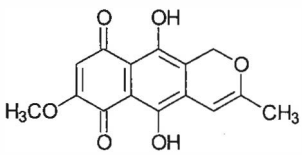
fusarubin-methyacetal



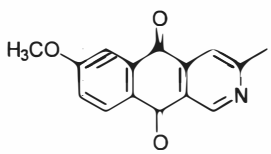
anhydro-5-deoxyfusarubin



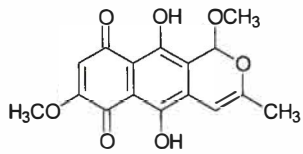
fusarubin



anhydrofusarubin



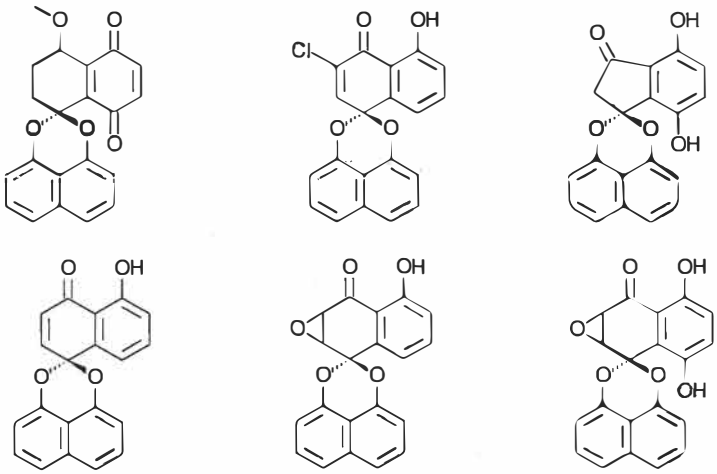
bostrycoidin



1-methoxy-anhydrofusarubin

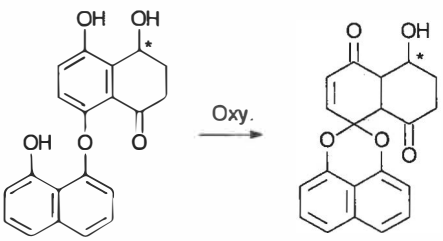
Finally, I shall mention a new group of polycyclic spiroacetals named palmarumycins isolated from *Coniothyrium* sp. and *Coniothyrium palmarum*.[8,9] (Fig. 9). The two strains are very productive. Over twenty different derivatives have been structurally elucidated from them. Interestingly, related structures have recently been isolated from different places araond the world (preussomerins[10-12] , diepoxins[13,14] and other related structures[15-17])

Figure 9. Sturctures of Selected Palmarumycins from *Coniothyrium palmarum*, Isolated from *Lamium purpureum*



The palmarumycins have recently been shown to be structurally derived by an oxidative coupling of two 1,8-dihydroxynaphthalene units as proposed in our publications[9]. K. Beckmann in our group isolated a derivative with a simple ether linkage between the two units which might be a possible biosynthetic intermediate. In fact, oxidation of this compound with silver oxide led to a spiro acetal related to the palmarumycins as shown in Fig. 10. More details of the palmarumycins can be seen on the poster presentation of K. Beckmann.

Figure 10. Oxidative Conversion of an Ether to the Palmarumycin Spiro Acetal



References

1) KROHN, K., C. FRANKE, P. G. JONES, H.-J. AUST, S. DRAEGER, & B. SCHULZ: Wirkstoffe aus Pilzen, I. Isolierung, Synthese und biologische Wirkung von Coniothyriomycin und analogen offenkettigen Imiden. Liebigs Ann. Chem.: 789-798, 1992

2) KROHN, K., K. LUDEWIG, P. G. JONES, D. DÖRING, H.-J. AUST, S. DRAEGER, & B. SCHULZ: Biologically Active Metabolites from Fungi, 2. A Novel Antifungal and Herbicidal Lanosterin Lactone from *Sporomiella australis*. Nat. Prod. Lett. 1: 29-32, 1992

3) KROHN, K., & C. KLICHE-SPORY. unpublishd results

4) SCHULZ, B., J. SUCKER, H.-J. AUST, K. KROHN, K. LUDEWIG, P. G. JONES, & D. DOERING: Biologically Active Secondary Metabolites of Endopyhtic Pezicula species. Mycolog. Res.: 1007-1015, 1995

5) KROHN, K., R. BAHRAMSARI, U. FLÖRKE, K. L., C. KLICHE-SPORY, A. MICHEL, H.-J. AUST, S. DRAEGER, B. SCHULZ, & S. ANTUS: Biologically Active Metabolites from Fungi, 8. Dihydroisocoumarins from Fungi: Isolation, Structure Elucidation, Circular Dichroism, Enantioselective Synthesis, and Biological Activity. Phytochemistry: submitted, 1996

6) KROHN, K., K. LUDEWIG, H.-J. AUST, S. DRAEGER, & B. SCHULZ: Biologically Active Metabolites from Fungi, 3. Sporothriolide, Discosiolide, and 4-Epi-ethiosolide - New Furofuranones from *Sporothrix* sp., *Discosia* sp. and *Pezicula livida*. J. Antibiot. 46: 113-118, 1994

7) KROHN, K., & R.. BARAMSARI. unpublishd results

8) KROHN, K., A. MICHEL, U. FLÖRKE, H.-J. AUST, S. DRAEGER, & B. SCHULZ: Biologically Active Metabolites from Fungi, 4. Palmarumycins CP_1 to CP_4 from *Coniothyrium palmarum*: Isolation, Structure Elucidation and Biological Activity. Liebigs Ann. Chem.: 1093-1097, 1994

9) KROHN, K., A. MICHEL, U. FLÖRKE, H.-J. AUST, S. DRAEGER, & B. SCHULZ: Biologically Active Metabolites from Fungi, 5. Palmarumycins C1 to C16 from

Coniothyrium sp.: Isolation, Structure Elucidation, and Biological Activity. Liebigs Ann. Chem.: 1099-1108, 1994

10) WEBER, H. A., & J. B. GLOER: The Preussomerins: Novel Antifungal Metabolites from the Coprophilous Fungus *Preussia isomera* Cain. J. Org. Chem. 56: 4355-4360, 1991

11) POLISHOOK, J. D., A. W. DOMBROWSKI, N. N. TSOU, G. M. SALITURO, & J. E. CUROTO: Preussomerin D from the Endophyte *Hormonema dematioides*. Mycologia 85: 62-64, 1993

12) SINGH, S. B., D. L. ZINK, J. M. LIESCH, R. G. BALL, M. A. GOETZ, E. A. BOLESSA, R. A. GIACOBBE, K. C. SILVERMAN, G. F. BILLS, F. PELAEZ, C. CASCALES, J. B. GIBBS, & R. B. LINGMAN: Preussomerins and Deoxypreussomerins: Novel Inhibitors of Ras Farnesyl-Protein Transferase. J. Org. Chem. 59, 1994

13) SCHLINGMANN, G., R. R. WEST, L. MILNE, C. J. PEARCE, & G. T. CARTER: Diepoxins, Novel Fungal Metabolites with Antibiotic Activity. Tetrahedron Lett. 34: 7225-7228, 1993

14) SCHLINGMANN, G., S. MATILE, N. BEROVA, K. NAKANISHI, & G. T. CARTER: Absolute Stereochemistry of the Diepoxins. Tetrahedron 52: 435-446, 1995

15) SAKEMI, S., T. INAGAKI, K. KANEDA, H. HIRAI, E. IWATA, T. SAKAKIBARA, Y. YAMAUCHI, M. NORCIA, L. M. WONDRACK, J. A. SUTCLIFFE, & N. KOJIMA: CJ-12,371 and CJ-12,372, Two Novel DNA Gyrase Inhibitors. Fermentation, Isolation, Structural Elucidation and Biological Activities. J. Antibiot. 27: 134-142, 1995

16) CHU, M., I. TRUUMEES, M. G. PATEL, V. P. GULLO, & M. PUAR: Structure of Sch 49209: A Novel Antitumor Agent from the Fungus *Nattrassia magniferae*. J. Org. Chem. 59: 1222-1223, 1994

17) THIERGARDT, R., P. HUG, G. RIHS, & H. H. PETER: Cladospirone Bisepoxide - A Novel Fungal Metabolite Structure Determination. Tetrahedron Lett. 35: 1043, 1994

Occurrence of the alkaloid lolitrem B in endophyte-infected *Lolium perenne*

OLDENBURG, E.

Institute of Grassland and Forage Research, Federal Agricultural Research Centre (FAL)
Braunschweig, Germany

ABSTRACT

The occurrence of lolitrem B in endophyte-positive ecotypes (origin: Germany) and the variety „Ellett" of *Lolium perenne* (origin: New Zealand) was examined throughout the vegetation periods of 1993 and 1994. The grasses were cultivated at an experimental field in Braunschweig-Völkenrode (FAL). Lolitrem B was determined by high performance liquid chromatography using fluorescence detection.

Both endophyte-infected German ecotypes and the cultivar „Ellett" gave positive results of lolitrem B. A seasonal variation of lolitrem B concentrations was observed achieving highest amounts of about 0.8 to 1.4 mg/kg dry matter of grass during the summer months.

These values are not sufficient for induction of „ryegrass staggers" in grazing animals, but may cause subclinical effects like reduced weight gains or lowered milk produc-tion.

Keywords: Endophyte, *Lolium perenne*, lolitrem B, ryegrass staggers

INTRODUCTION

The presence of endophytic fungi in European ecotypes and varieties of *Lolium* and *Festuca* grasses was confirmed by several studies carried out in the United Kingdom, France, Switzerland, Czech Republic and Germany during the last 10 years (Lewis and Clements, 1986; Mika, 1990; Grand-Ravel et al., 1993; Pfannmöller et al., 1994; Dapprich et al., 1994). However, there is only little information available so far on the

occurrence of alkaloids produced in these infected grasses under the climatical conditions of Central Europe.

In New Zealand, Australia and USA the enrichment of alkaloids in endophyte-positive perennial ryegrass and tall fescue pastures is well documented. These alkaloids may have beneficial effects on the resistance of grasses against microbial pathogens, insects and nematodes, but detrimental effects on the health and performance of grazing animals like cattle, sheep, horses and deer (Prestidge and Gallagher, 1989; Galey et al., 1991; Van Heeswijck and McDonald, 1992; Siegel, 1993).

The indole alkaloid lolitrem B is produced in perennial ryegrass naturally infected with the endophytic fungus *Acremonium lolii*, but can also be found in other grass species, for example tall fescue, that was artificially infected with *Acremonium lolii* and hard fescue naturally infected with *Epichloe typhina* (Siegel et al., 1990). Lolitrem B can cause a neurological disorder in livestock named „ryegrass staggers" which occurs in New Zealand and parts of the USA. In Europe it was only sporadically observed until now, for example in the U.K. and the Netherlands (Lewis and Clements, 1986; Fink-Gremmels and Blom, 1993). Clinical symptoms may range from slight muscle tremors to severe staggering, lack of coordination, collapse and muscular spasms (Prestidge, 1993; Rowan, 1993; Keogh, 1973).

In order to get more knowledge on the production of alkaloids in grass-endophyte associations occurring on indigenous grassland enrichment of lolitrem B in endophyte-positive *Lolium perenne* was studied at a location in Germany throughout the vegetation period.

MATERIALS AND METHODS

Plant material

Endophyte-positive ecotypes of *Lolium perenne* (origin: Germany) and the variety „Ellett" of *Lolium perenne* (origin: New Zealand) were cultivated at an experimental field in Braunschweig-Völkerode (FAL). The overground biomass of the ecotypes, grown as single plants, was cut 4 times during April to December in 1994. The cultivar „Ellett" was sown both as endophyte-positive variant (infection rate >80%; 3 plots of 12.6 m^2) and as endophyte-negative variant (1 plot of 12.6 m^2). During May to October in 1993 and 1994 the overground biomass of the variants was cut 4 to 5 times, respectively, by using a plot

harvester. All harvested materials of ecotypes and the variety „Ellett“ were chopped to an average length of 5 mm. Representative amounts of chopped biomass were freeze-dried, ground to a fineness of 1 mm and analysed for lolitrem B.

Determination of lolitrem B

Lolitrem B was determined by high performance liquid chromatography using flourescence detection. Grass extraction, extract clean-up and chromatographic conditions were applied according to the method of Gallagher et al. (1985). The HPLC-system 450 (Kontron Instruments) was used with a Lichrosorb Si 60-column (250 x 4 mm, particle size 5 µm; Merck) and the flourescence detector SFM 25 (Kontron Instruments). The excitation wavelength was set at 268 mn and the emission wavelength at 440 nm. Lolitrem B was identified by retention time of standard and quantified by standard calibration curve (10 to 1000 ng/ml) of peak area.

RESULTS

Lolitrem B was accumulated in all endophyte-infected German ecotypes grown in 1994 (Table 1). Single plants cut in June showed similar mean values of about 509 to 575 µg lolitrem B/kg dry matter despite of different geographical origin of the grasses, but a great variation of lolitrem B levels was observed in individual plants (199 - 1430 µg/kg DM).

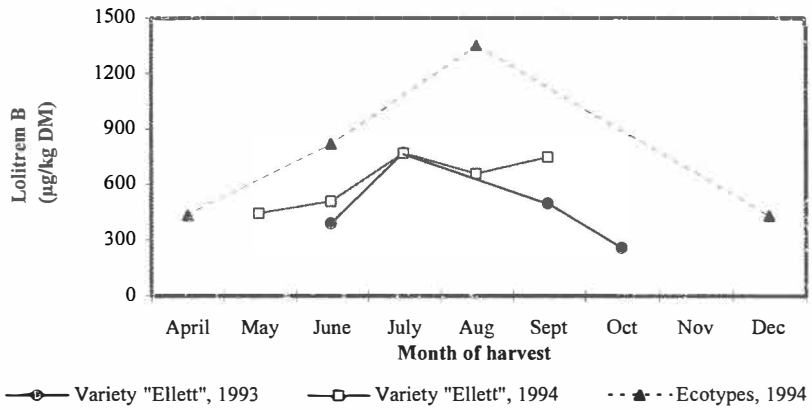
Table 1: Concentration of lolitrem B in ecotypes of *Lolium perenne*
Biomass of single plants; Time of harvest: 08.-22.06.1994

Region of provenance	Single plants n	Lolitrem B (µg/kg DM) x	Range
Lower Saxony	11	575	199 - 810
Hesse	18	505	251 - 1430
Island of Poel	8	509	332 - 672

During the vegetation periods of 1993 and 1994 seasonal differences in lolitrem B enrichment were found both in endophyte-positive variant of „Ellett“ and in German

ecotypes of *Lolium perenne* (Figure 1). The lolitrem B contents in endophyte-infected „Ellett" (average values of harvest material from 3 plots) increased from 300-400 µg/kg DM in late spring to maximum values of about 700-800 µg/kg DM in late summer and decreased afterwards to 300 µg/kg DM in October. In endophyte-negative variant of „Ellett" no positive result of lolitrem B occurred at any time of the year. In German ecotypes a clearly higher level of lolitrem B (mean concentrations of 4 endophyte-infected single plants) was also observed in summer as compared to spring and winter.

Figure 1: Seasonal levels of lolitrem B in *Lolium perenne*



DISCUSSION

This study proved that production of lolitrem B is common in endophyte-infected perennial ryegrass when growing under the climatical conditions of Central Europe. In endophyte-positive *Lolium perenne* ecotypes originating from German grassland similar levels of lolitrem B were achieved as compared to the endophyte-infected variant of the cultivar „Ellett" from New Zealand, but lolitrem B accumulation varied to a greater extent in single plants. It is concluded that environmental conditions at the location of growth and specific parameters of individual grass/endophyte associations (genetic diversity, amount of mycelium in the plant) may influence the intensity of alkaloid production rather than the geographical provenance of the grasses.

The observed seasonal variations of lolitrem B concentrations agree with findings from New Zealand, where maximum values also occur during the summer months

(Prestidge and Gallagher, 1989; Ball et al., 1991). But in comparison with data derived from other countries, maximum lolitrem B concentrations found in this study were 3 to 10 times lower than those detected in New Zealand, USA, Great Britain, where highest values ranged between 3 and 10 mg/kg DM of grasses (Prestidge and Gallagher, 1988; Siegel et al., 1990; Ball et al., 1991; Galey et al., 1991; Lewis and Clements, 1986).

Lolitrem B has to be enriched to at least 2 mg/kg DM of grass to induce clinical symptoms of „ryegrass staggers" (Prestidge and Gallagher, 1989; DiMenna et al., 1992). As the level of lolitrem B observed in our study varied in a considerable lower range (0.2 - 1.4 mg/kg DM), an outbreak of „ryegrass staggers" is assumed to be rather unlikely in Germany. However, it should be clarified, if an intake of low contaminated grasses may lead to subclinical effects like reduced weight gains, lowered milk production, lowered fat and protein content of the milk and reduced hormonal levels in livestock (Prestidge, 1993; Valentine et al., 1993).

Acknowledgements

The author thanks Dr. Ian Garthwaite, Ruakura Agricultural Centre, Hamilton, New Zealand, for the generous gift of lolitrem B standard and Karin Trescher for excellent technical assistance.

REFERENCES

Ball, O.J.-P.; Prestidge, R.A.; Sprosen, J.M. and Lauren, D.R. 1991. Seasonal levels of peramine and lolitrem B in *Acremonium lolii*-infected perennial ryegrass. Proc. 44th N.Z. Weed and Pest Control Conference, 176-180.

Dapprich, P.; Klose, A. and Paul, V.H. 1994. A survey on the occurrence of the endophytic fungi in European seed lots of *Lolium perenne*. Proceedings of the International Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf, Paderborn, 4-6 October 1993, Krohn K.; Paul, V.H. and Thomas, J. (eds.), IOBC/WPRS Bulletin, Bulletin OILB/SROP Vol. 17 (1), 131-137.

DiMenna, M.E.; Mortimer, P.H., Prestidge, R.A., Hawkes, A.D. and Sprosen, J.M. 1992. Lolitrem B concentrations, counts of *Acremonium lolii* hyphae, and the

incidence of ryegrass staggers in lambs on plots of *A. lolii*-infected perennial ryegrass. N.Z. Agr. Res. 35, 211-217.

Fink-Gremmels, J. and Blom, M. 1993. Neurotoxische Mykotoxine in Weidegras. Berichtsband 15. Mykotoxin-Workshop, 17.-19.5. 1993, Jena.

Galey, F.D.; Tracy, M.L.; Craigmill, A.L., Barr, B.C.; Markegard, G.; Peterson, R. and O'Connor, M. 1991. Staggers induced by consumptions of perennial ryegrass in cattle and sheep from northern California. Journal of the American Veterinary Medical Association 199 (4), 466-470.

Gallagher, R.T.; Hawkes, A.D. and Stewart, J.M. 1985. Rapid determination of the neurotoxin lolitrem B in perennial ryegrass by high performance liquid chromatography with flourescence detection. Journal of Chromatography 32, 217-226.

Grand-Ravel, C.; Charmet, G. and Balfourier, F. 1993. A quick survey of *Acremonium* endophyte in breeding populations of perennial ryegrass. Proceedings of the Second International Symposium on *Acremonium*/Grass Interactions, Hume, D.E.; Latch, G.C.M. and Easton, H.S. (eds.), AgResearch, Grasslands Research Centre, Palmerston North, New Zealand, 205-209.

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

Lewis, G.C. and Clements, R.O. 1986. A survey of ryegrass endophyte (*Acremonium loliae*) in the U.K. and its apparent ineffectuality on a seedling pest. Journal of Agricultural Science, Camb., 107, 633-638.

Mika, V. 1990. Endophytenprobleme bei Futtergräsern. Tagungsbericht der 32. Fachtagung des Ausschusses für Gräser, Klee und Zwischenfrüchte, 5.-6.12. 1990, Fulda, 123-129.

Pfannmöller, M.; Eggestein, S. and Schöberlein, W. 1994. Endophytes in European varieties of *Festuca* species. Proceedings of the International Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf, Paderborn, 4-6 October 1993, Krohn, K.; Paul, V.H. and Thomas, J. (eds.), IOBC/WPRS Bulletin, Bulletin OILB/SROP Vol. 17, 105-109.

Prestidge, R.A. and Gallagher, R.T. 1988. Endophyte fungus confers resistance to ryegrass: Argentine stem weevil larval studies. Ecological Entomology 13, 429-435.

Prestidge, R.A. and Gallagher, R.T. 1989. *Acremonium* endophyte in perennial ryegrass, ryegrass staggers in lambs, and growth of Argentine stem weevil larvae. Proceedings 5th Australian Conference on Grassland Invertebrate Ecology, 15-16 August 1988, Melbourne, Stahle, P.P. (ed.), Vic. D&D Printing Pty., Victoria, Australia, 229-235.

Prestidge, R.A. 1993. Causes and control of perennial ryegrass staggers in New Zealand. Agriculture, Eco-systems and Environment 44, 283-300.

Rowan, D.D. 1993. Lolitrems, peramine and paxilline mycotoxins of the ryegrass/endophyte interaction. Agriculture, Ecosystems and Environment 44, 103-122.

Siegel, M.R.; Latch, G.C.M.; Bush, L.P.; Fannin, F.F.; Rowan, D.D., Tapper, B.A.; Bacon, C.W. and Johnson, M.C. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. Journal of Chemical Ecology 16 (12), 3301-3315.

Siegel, M.R. 1993. *Acremonium* endophytes: our current knowledge and future directions of research. Agriculture, Ecosystems and Environment 44, 301-321.

Valentine, S.C.; Bartsch, B.D. and Caroll, P.D. 1993. Production and composition of milk by dairy cattle grazing high and low endophyte cultivars of perennial ryegrass. Proc. 2nd International Symposium on Acremonium/Grass Interactions, 138-141.

Van Heeswijck, R. and McDonald, G. 1992. *Acremonium* endophytes in perennial ryegrass and other pasture grasses in Australia and New Zealand. Australian Journal of Agricultural Research 43, 1683-1709.

Incidence of *Acremonium* endophytes in selected German pastures and the contents of alkaloids in *Lolium perenne*.

Peter DAPPRICH[a], Volker H. PAUL[a], and Karsten KROHN[b]

[a] Universität-Gesamthochschule-Paderborn, Fachbereich Landbau, Lübecker Ring 2, 59494 Soest

[b] Universität-Gesamthochschule-Paderborn, Fachbereich Chemie und Chemietechnik, Warburger Straße 100, 33098 Paderborn

Abstract

Endophytic fungi can be present in a number of European seed samples of *Lolium perenne*, as we proved in recent studies (DAPPRICH et al., 1994a). In this collective spot check, however, no *Acremonium* was found in German seeds.

Other authors (OLDENBURG, 1994) proved, that endophytic fungi of the genus *Acremonium* are present in *Lolium* spp., found in German grassland in the states of Lower Saxony, Hesse, Bavaria, and the island of Poel. In this paper we report the presence of *A. lolii* in *L. perenne*-samples collected from old „low input" pastures in the Duisburg area in the state of Northrhein Westfalia. The infection rates of the plant samples ranged between 19 and 40 %. Cattle grazing on those pastures showed health disorders resembling the „ryegrass staggers syndrome" reported by different authors from New Zealand, Australia and the United States (FLETCHER et al., 1990; KEOGH 1986; WELTY et al., 1987) .

The analysis of grass samples containing alkaloids proved the presence of lolitrem B, perloline and hints of peramine. With the analysing techniques used however, the presence of lolines was not demonstrable. From these grass samples several isolates of *Acremonium* were gained and multiplied using biotechnological methods. These isolates were examined for their ability to build different alkaloids biosynthetically *in vitro*. Other recent studies showed that *Acremonium* is able to build a number of alkaloids *in vitro* when influenced by precursors.

Keywords: *Acremonium lolii*, German low input pastures, lolitrem B, ryegrass staggers, alkaloid biosynthesis *in vitro*

Introduction

Lolium perenne is the most important fodder grass in Germany and in most parts of Europe. It has a share of more than 50-70 % in intensively used forage fields. In a screening for endophytic fungi of European *Lolium perenne* seeds carried out by our group in 1993/94 a number of endophytic fungi of the genus *Acremonium* were found (AHMAD et al., 1986; DAPPRICH et al., 1994a). As reports from the USA and New Zealand show, these fungi can induce the production of resistance promoting secondary metabolites of the group of the alkaloids in their hosts. Under certain environmental conditions however, the production of toxins is possible which can have negative effects on livestock. So far there have been no reports of seedborn fungi in German *Lolium* seeds. Only in German *Festuca* cultivars *Acremonium* was detected (PFANNMÖLLER et al., 1994). Nevertheless, *Acremonium* is present in German grassland and seems to be widely spread (OLDENBURG, 1994). Due to reports of grazing young cattle showing symptoms resembling those of "ryegrass staggers" in the summer of 1994 (July / August), samples of those pastures were taken by our group. In the plant samples of *Lolium perenne* we showed the presence of *Acremonium*-endophytes.

It is the purpose of the study at hand to present information about the existence of *Acremonium* in German pastures and first information concerning the presence of alkaloids in *Lolium* under German field conditions. Furthermore, we shall discuss the possibility of influencing the biosynthesis of alkaloids in *Acremonium in vitro.*

Materials and Methods

Four pastures in the area of Duisburg in Northrhein Westfalia on which abnormal behaviour of grazing cattle was noticed, were examined for their endophyte contents in *Lolium perenne* grasses. The samples were taken by pacing off the pastures in parallel lanes approximately 3 m apart. Along these lanes *Lolium* plant samples were taken at about every 3 m. These plant samples were collected in an ice box and transported to the

laboratory for examination for their endophyte contents (WELTY et al., 1986; DAPPRICH et al., 1994b). The complete biomass obtained was divided into two sections, a leaf sheath (1-5 cm long) and a leaf blade (5-x cm) section. These sections were freeze dried, ground in a hammermill (screen diameter 1 mm) and separately prepared for the alkaloid analysis. For the determination of the *Lolitrem B* contents one gram of each section was prepared according to GALLAGHER (1985) and analysed using HPLC. This analysis was carried out under the guidance of Dr. Oldenburg at the Institute of Grassland and Forage Research, Federal Agricultural Research Centre (FAL) in Braunschweig. We want to thank Dr Oldenburg at this place for her friendly support.

For the examination of lolines (pyrrolizidine-alkaloids) and perloline, 10 grams of the respective samples were extracted over night with 400 ml of methanol in a Soxhlett-Extractor. The organic solution was evaporated at 40°C and the residue resuspended in 100 ml 1 N hydrochloric acid. The aqueous phase was washed three times with 50 ml of ethyl acetate and one time with 50 ml chloroform. The organic phases were rejected. The aqueous phase was adjusted to a pH of >10 with 30 ml of a 25% ammonium hydroxide solution. These basic solution was extracted three times with 50 ml chloroform, the organic phases were combined, dried over sodium sulphate and then evaporated at 30°C to dryness. The residue was resuspended in one ml chloroform.

The crude extract was separated into its components by using high performance thin layer chromatography glass-plates coated with silica gel (HPTLC, 10 x 10 cm, Merck, G 60, Art.No. 5631). For separation we used chloroform, methanol and 25% ammonium hydroxide solution (75 : 24 : 1) as a mobile phase. Detection of the alkaloids was performed by dipping the plates for a few seconds into Dragendorff solution.

The isolation of the endophytic fungi from the plant samples was a modification of the method described by LATCH & CHRISTENSEN (1985). Single tillers of endophyte positive plant samples were cleaned, the leaf blade removed and the tillers dipped in 70% isopropanol for 30 sec. Subsequently they were surface sterilised in 10% sodium hypochloride (NaOCl) for 10 minutes. After washing in sterile water the tillers were cut into 5-7 mm long pieces and placed on PDA in 9 cm Ø plastic petri dishes sealed with

parafilm. Then they were incubated at 22°C in the dark. The identification of the isolates occurred in accordance to LATCH et al., (1984).

The biotechnological biomass production of the isolated endophytic fungi followed the description of DAPPRICH et al. (1995 b).

In earlier experiments cultures of *Acremonium lolii*, *A. coenophialum* and *Epichloë typhina* obtained from CBS (Centraalbureau voor Schimmelcultures, Baarn, NL) were cultivated in a 2.5 l "Airlift-Bioreactor" (Fig. 1). To ensure that the bioreactor would not clog up, the endophytes were immobilised in sodium alginate (RHODES, 1985). For this purpose 10 ml of a homogenised endophyte suspension was mixed with 100 ml sodium alginate (5% in PDB), blended and dripped into a 2% calciumchloride-solution. The resulting balls were transferred into the bioreactor in PDB (2.5 l total volume) and cultivated at 23°C with air circulation. After the alginate balls were grown through completely, the biomass was harvested, freeze dried and like the culture medium, tested for the presence of alkaloids by means of HPTLC.

To examine the possibilities of influencing the alkaloid-biosynthesis of endophytic fungi in vitro spermidine, putrescine and 1.4-diamino-butanone (10 mg/l each) was added to the cultures in the bioreactor in different experiments. Spermidine and putrecine are precursors in the pyrrolizidine-alkaloid biosynthesis in *Symphytum* (HUIZING, 1983). 1.4-diamino-butanone is a structural analogue of putrescine.

Results

The survey of the pastures in the Duisburg area showed a high contents of endophyte-infected *Lolium* plants. The maximum contents reached up to 40% (pasture I). Those pastures contained *Lolium* in amounts estimated between 50% and more than 70% (Table 1).

Figure 1 Structure of an „Airlift-Bioreactor“ for the *in vitro* culture of *Acremonium lolii*

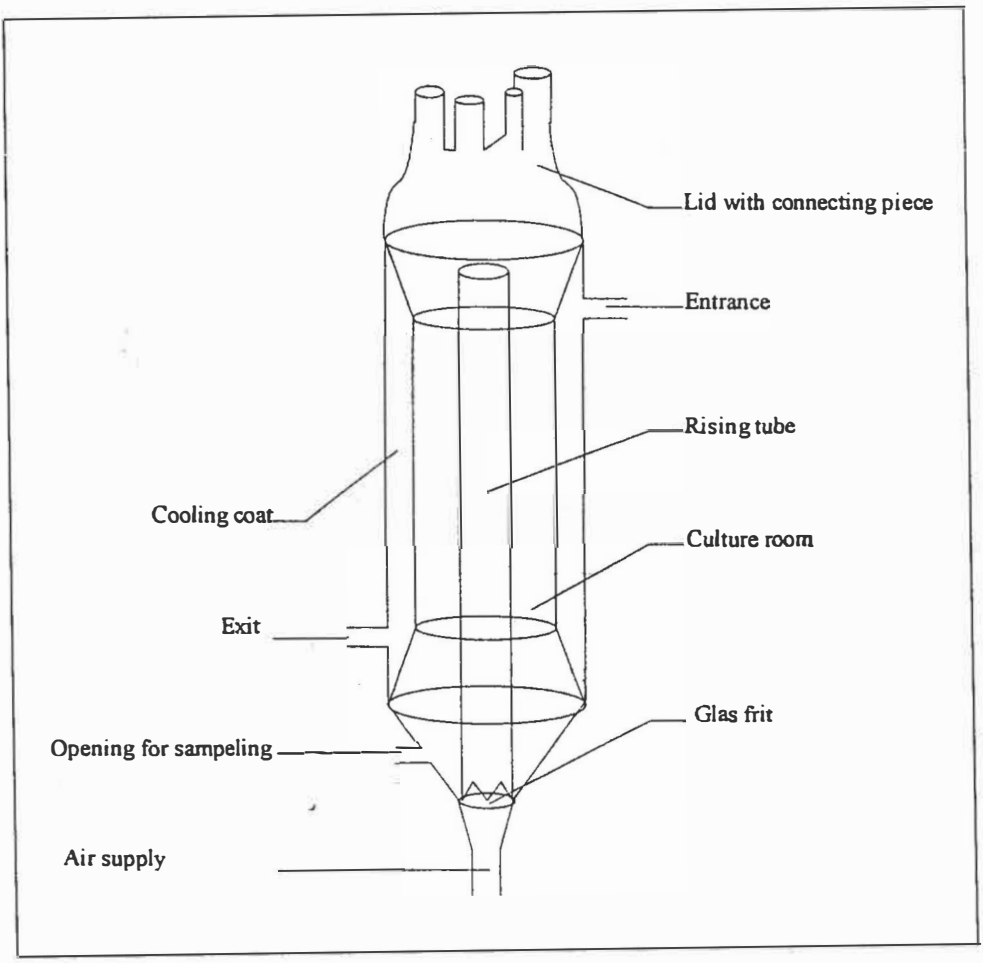


Table 1 Occurrence of the endophytic fungi *Acremonium lolii* in *Lolium perenne* on long term „low input" pastures in the state of Northrhein Westfalia (Duisburg area) in the year 1994

Pasture No.	Area [m²]	Estimated contents of Lolium perenne [%]	Number of samples	Number of endophyte positive plantlets (E(+))	Percentage of E (+) [%]
I	ca. 750	> 70 %	88	35	39,7
II	ca. 500	> 50 %	53	10	18,8
III	ca. 500	> 60 %	47	14	29,7
IV	ca. 400	> 70 %	37*	3	8,1

* no representative spot check

The obtained isolates of the drawn samples were all identified as *Acremonium lolii*. Other endophytic fungi of the *Balansia*-group were not found.

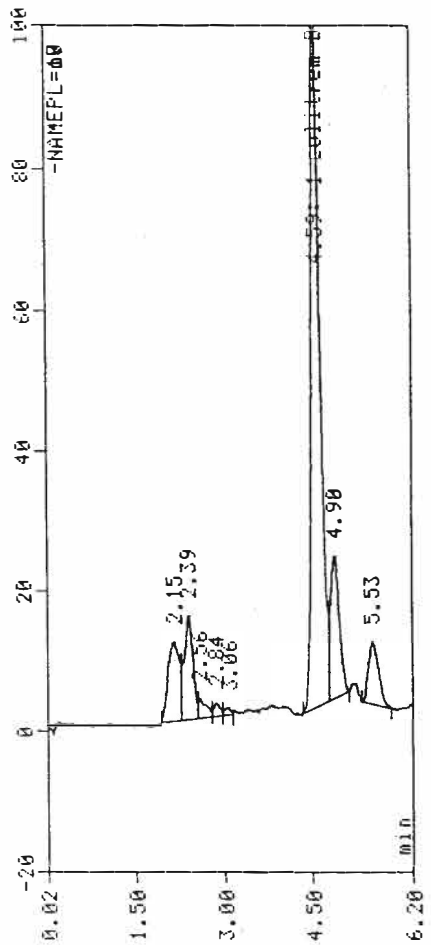
The first qualitative examination of the biomass on lolitrem B showed a high contents in the leaf sheath section and nearly 80 % lower contents in the leaf blades (Fig. 2). The lolitrem B peak appeared after about 4.6 minutes at the chosen conditions.

In our earlier work the TLC was used to separate and identify perloline and lolines, as described also in literature (SACHSE, 1980; MOLYNEUX & ROITMAN, 1980). In our own tests it was possible to prove that alkaloids were present as well in the biomass as in seed samples of endophyte-infected *Lolium perenne* and *Festuca pratensis* material. The plant material used originated from field and greenhouse trials. A difference in the alkaloid contents and alkaloid pattern between field and greenhouse trial at high average temperatures (> 25°C) was not distinguishable. In most of the chosen grass types differences in the contents and position of alkaloid spots between endophyte positive and endophyte negative showed clearly. The cultivars Pennant and Repell showed an increase of Dragendorff-detectable alkaloids due to the influence of endophytes, Ellet did not. The

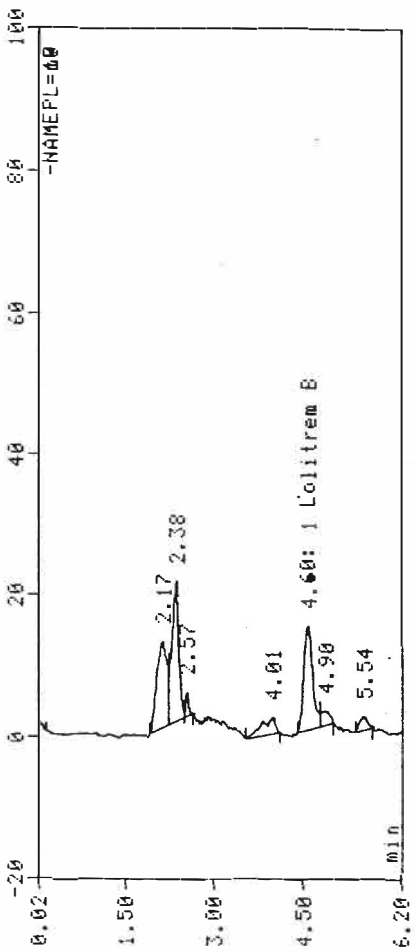
samples of *Lolium* and *Festuca* showed clear differences in amount and position of alkaloid spots (DAPPRICH et al., 1994c).

Figure 2 HPLC-chromatograms of lolitrem B in *Lolium perenne* on long-term „low input" pastures in the area of Duisburg (Federal State of Northrhein-Westfalia) in the year 1994

a) Leaf sheath section



b) Leaf blade section

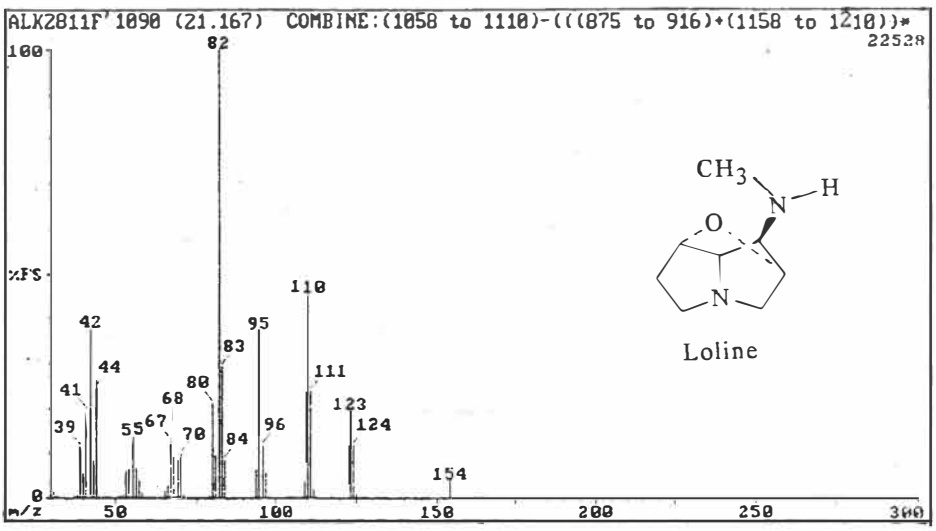


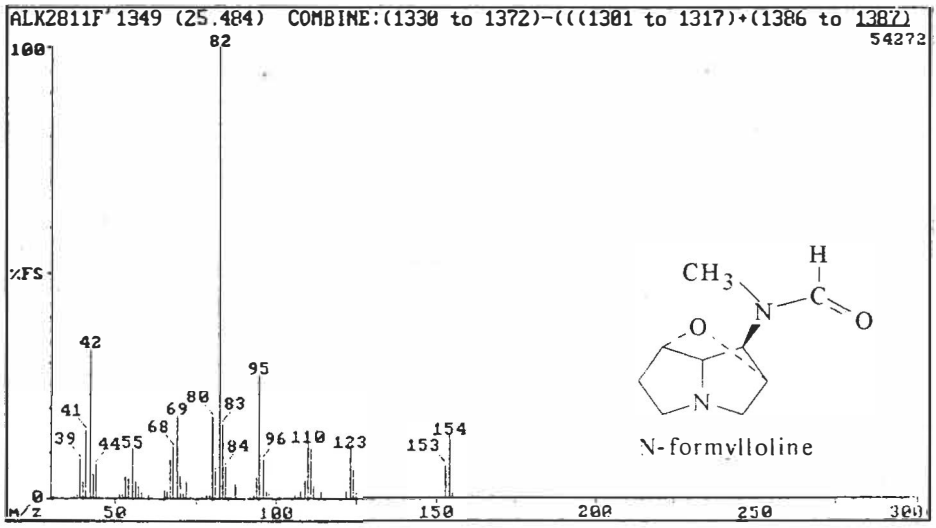
In the field samples we screened no pyrrolizidine-alkaloids could be found. Nevertheless perloline was found in accordance to the reports of SACHSE (1980) with a high probability.

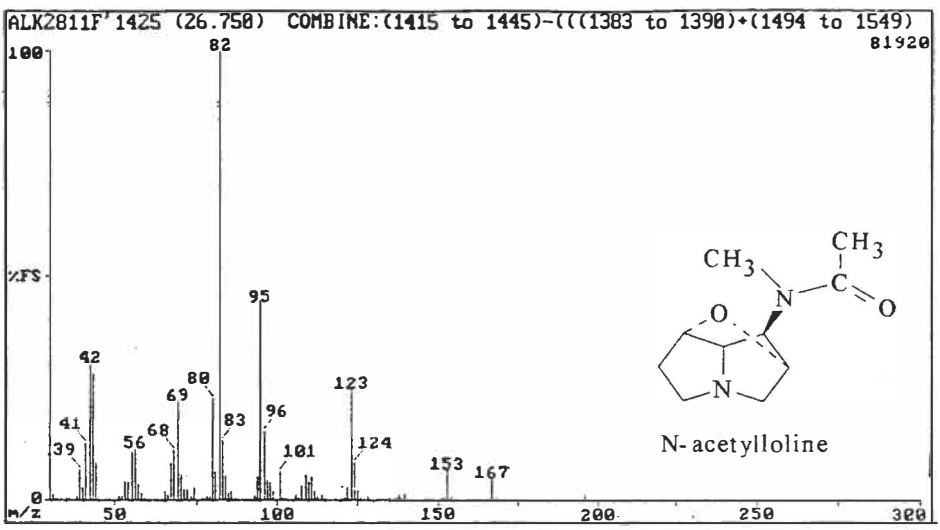
A final statement in our opinion is only possible after exact identification with other methods, e.g. with GC-MS. As our test results show this method is also suitable for the qualitative proof of peramine (TAPPER et al., 1989).

A GC-MS examination of the isolated alkaloids of seeds and biomass of *F. pratensis* showed loline, N-formylloline and N-acetylloline being present in the samples (Fig. 3) along with at least one yet unidentified alkaloid. With the help of GC-MS lolines were found in seed extracts of *Lolium perenne*, too, but not in plant material. The proof of loline in *Lolium* with DC was neither possible in seed extracts nor in biomass.

Figure 3 Total ion spectra of loline, N-formylloline and N-acetylloline, respectively







As for the endophytes themselves in our analysis we could not confirm the biosynthesis of any ergot-alkaloids by *Acremonium* in Erlenmeyer-flasks in vitro influenced by selection of specific media as described by BACON (1988).

Therefore, the culture volume was raised with the use of the bioreactor (Fig. 1) to gain possibly higher amounts of alkaloids by higher biomass production. The growth parameters were generally transferable from the Erlenmeyer-flasks to the culture in the bioreactor if the growth of the fungi was not hindered with a too strong airstream or

uneven temperature control. With the use of precursors of the pyrrolizidine-alkaloid biosynthesis it was tried to stimulate their biosynthesis *in vitro*. The use of putrescine and spermidine as well as 1.4-diamino-butanone showed that a stimulation of alkaloid-biosynthesis *in vitro* is quite possible. However, this succeeded only in one of two tested CBS isolates of *A. lolii*. Here, it was possible to raise the amount of TLC distinguishable alkaloids from one to nine. An identification of these substances is in progress. Loline or one of it's derivatives could not be proved. With *Acremonium coenophialum* and *Epichloë typhina*, the stimulation of the alkaloids biosynthesis was not possible. The co-cultivation of *A. lolii* and *L. perenne*-cells in alginate showed the synthesis of other so far not identified alkaloids. With the addition of precursors to this system the alkaloid-biosynthesis with this methods was also clearly increased. Identification of these substances has not yet taken place.

Discussion

Our examination of specific grasslands in Northrhein-Westfalia confirm the results of Dr. OLDENBURG (1994) from other federal states. Accordingly, endophytes of the genus *Acremonium* could be spread on pastures all over Germany. The abnormalities in behaviour of cattle grazing on *Acremonium*-infected pastures reported to us indicate in their symptomatology "ryegrass staggers syndrome". This makes the presumption obvious that such abnormal behaviour as reported from the USA, New Zealand and Australia, could not only appear in our climatic zones but could also have considerable impact on livestock health. We think that further examinations are urgently required.

The *in vitro* examinations showed that it is possible to influence the alkaloid biosynthesis not only through specific selection of culture-media, as described by BACON (1988), but also through employment of certain precursors or other chemical substances. An exact analysis of the build substances can give on one side general information on the biosynthesis of pyrrolizidine-alkaloids in *Acremonium* and, on the other hand, a possibility for the in vitro synthesis of desired alkaloids. Furthermore, this could be developed into a method for testing endophytes *in vitro* in view of their suitability in breeding programs for new grass varieties and artificial infection trials for the specific improvement of existing cultivars. The results of those tests could complement, or in

some areas even replace, the genetechnological work with endophytes and/or grasses. A quick progress in this work is therefore urgently needed.

Acknowledgements

We are grateful to the Federal Ministry of Food, Agriculture and Forestry (Bundesministerium für Ernährung, Landwirtschaft und Forsten (BML)), and the Society for Sponsering of Private German Plant Breeding (Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e. V. (GFP)) for their financial support.

References

Ahmad, S., Johnson-Cicalese, J.M., Dickson, W.K., and Funk, C.R. (1986). Endophyte-enhanced resistance in perennial ryegrass to the bluegrass billbug, *Shenophorus parvulus*. Entomol.exp.appl., 41:3-10

Bacon, C.W. (1988). Procedures for isolating the endophyte from tall fescue and screening isolates for ergot alkaloids. Appl.Environm.Microbiol., 54(11): 2615-2618

Dapprich, P., Klose, A. and Paul, V.H. (1994a). A survey on the occurrence of endophytic fungi in European seed lots of *Lolium perenne*. IOBC wprs Bulletin, 17(1): 131-137

Dapprich, P., Paul, V.H., and Krohn, K. (1994b). A novel and rapid staining method for the detection of vital endophytes in seeds and leaf sheaths of *Lolium perenne*. IOBC wprs Bulletin, 17(1): 139-146

Dapprich, P. and Paul, V.H. (1994c). Preliminary results on the detection of some characteristic alkaloids of *Acremonium* spp. in *Lolium perenne* in Germany. IOBC wprs Bulletin, 17(1): 181-186

Dapprich, P., Paul, V.H., and Krohn, K. (1995b). First results on the isozyme variation of isolates of *Acremonium* spp. from German pastures. in Press.

Gallagher, R.T., Hawkes, A.D., and Steward, J.M. (1985). Rapid determination of lolitrem B in perennial ryegrass by high-performance liquid chromatographie with fluorescence detection. J.Chromatogr. 132: 217-226

Huizing, H.J. (1983). Phytochemistry, systematics and biogenesis of pyrrolizidine alkaloids of *Symphytum officinale* taxa. Ph.D.thesis of Groningen

Keogh, R.G. (1986). Fungal distribution and livestock defoliation patterns in pasture ecosystems, and the development and control of dietary-dependent disorders. Proc.N.Z.Grass.Assoc., 47: 93-98

Latch, G.C.M., Christensen, M.J. and Samuels, G.J. (1984). Five endophytes of *Lolium* and *Festuca* in New Zealand. Mycotaxon, XX(2): 535-550

Latch, G.C.M. and Christensen, M.J. (1985). Artificial infection of grasses with endophytes. Ann.appl.Biol., 107: 17-24

Molineux, J.R. and Roitman, J.N. (1980). Specific detection of pyrrolizidine alkaloids on thin-layer chromatograms. J.Chromatography, 195: 412-415

Oldenburg, E. (1994). Occurrence of fungal endophytes in *Lolium perenne*. IOBC wprs Bulletin, 17(1): 99-104

Pfannmöller, M., Eggestein, S., and Schöberlein, W. (1994). Endophytes in European varieties of *Festuca* species. IOBC wprs Bulletin, 17(1): 105-109

Rhodes, M.J.C. (1985). Immobilised plant cell cultures. Topics in Enzyme and Fermentation Biotechnology, 10: 50-87

Sachse, J. (1980). Perlolinbestimmung in Gräsern. J.Chromatogr. 192: 199-207

Tapper, B.A. and Rowan, D.D. (1989). Detection and measurement of the alkaloid peramine in endophyte-infected grasses. J.Chromatogr. 463: 133-138

Welty, R.E., Milbrath, G.M., Faulkenberry, D., Azevedo, M.D., Meek, L., and Hall K. (1986). Endophyte detection in tall fescue seed by staining and ELISA. Seed Sci. & Technol., 14: 105-116

Content of perloline and water soluble carbohydrates in *festuca pratensis* (Huds.) plants infected by *Bipolaris* and *Drechslera* pathogens

Malgorzata Horodecka

Plant Breeding and Acclimatization Institute
Department of Forage Crops, 30-438 Krakow, Poland

ABSTRACT

Investigations were carried out to determine the reaction of *Festuca pratensis* plants on infection by 4 pathogens: *Bipolaris sorokiniana, Drechslera dictyoides, D. siccans* and *D. avenae.* 20 genotypes and 1 variety - Skrzeszowicka of meadow fescue were infected by a mixture of mycelium fragments and spores of the 4 pathogens mentioned above. 2 weeks after infection plants were collected and stored at -20°C until analyzed. Before analysis, plant material was dried at about 50°C and examined for the content of perloline and water soluble carbohydrates.

The control plants indicated a great genetic variability in the production of perloline. The content of perloline in this group of plants ranged from 0.84 to 37.56 mg/100 g d.m.. However, the highest level of perloline occured in the plants with strong disease symptoms (i.e. the spotting of leaves): from l. 81 to 62.54 mg/100 g d.m. (average 17. 31 mg/100 g d.m.), whereas the plants without disease symtoms contained considerably less perloline: from 1.29 to 39.12 mg/100 g d.m. (average 7.06 mg/100 g d.m.).

The level of water soluble carbohydrates in the plants without disease symptoms was mostly higher than that in the other 2 groups of plants (i.e. the group with disease symptoms and the group of control plants) and amounted on average to 18.09%. The average content of water soluble carbohydrates in the 2 groups of plants mentioned above was respectively:14.41% and 15.30%. It seems that the significantly low level of perloline in the plants without disease symptoms may be connected with the high level of water soluble carbohydrates in this group of plants. This observation requires further research.

Keywords: *Drechslera* spp., *Bipolaris sorokiniana, Festuca pratensis,* alkaloids, perloline, water soluble carbohydrates.

INTRODUCTION

Perloline is the main alkaloid of fescue species (*Festuca* L., spp.) and since it may have physiological effects on ruminants (Cunningham et al. 1943; Grimmett et al. 1943; Reifer et al. 1943), many investigations have been carried out to determine the factors which control its concentration in fescue plants.

The content of perloline in the plants is related to season, genotype and the amount of applied nitrogen and potassium. Many previous studies have proved that the level of perloline in endophyte-infected plants is higher than that in noninfected plants (Yates et al.1989; Renner 1987).

The purpose of this investigation was to determine the content of perloline in meadow fescue plants infected by *Bipolaris* and *Drechslera* pathogens. Simultaneously, the content of water soluble carbohydrates in these plants was determined because it seems that it may be connected with plant physiological mechanisms in stress conditions, such as an infection by pathogenic fungi.

MATERIALS AND METHODS

1. MATERIALS

Fungi: 4 pathogens of *Festuca pratensis* (Huds.): *Bipolaris sorokiniana* (Sacc.) Shoem., *Drechslera dictyoides* (Drechsl.) Shoem., *D. siccans* (Drechsl.) Shoem., *D. avenae* (Eidam.) Scharif

Plants: 20 genotypes and 1 variety - Skrzeszowicka of *Festuca pratensis* (Huds.).

2. METHODS

The pathogens were incubated separately in Petri dishes with the PDA medium under nuv. 4 weeks later the water mixture of mycelium and spores of the 4 pathogens was prepared. Meadow fescue plants growing in pots for the experiment were infected, in the 7th week after sowing, by a mixture of mycelium fragments and spores of the 4 pathogens mentioned above. The fertilizer treatment was: 60 kg N/ha, 80 kg P_2O_5/ha,

50 $K_2$0/ha. Plants were cut off to a height of 5 cm two weeks after infection (in the middle of July) and stored at -20°C until analyzed. Before analysis, plant material was dried at about 50°C and ground to pass 3-mm screen. The content of water soluble carbohydrates in the plant material was determined by the fluorometric method (using o-diacetylochromotropic acid, disodium salt), while the content of perloline was determined by uv-spectrophotometry (Mendelewski et al. l985, Yates et al. 1975).

TAB.I. CONTENT OF PERLOLINE AND WATER SOLUBLE CARBOHYDRATES IN FESTUCA PRATENSIS (HUDS.) PLANTS INFECTED BY BIPOLARIS AND DRECHSLERA PATHOGENS.

NR OF GENO-TYPE	PERLOLINE [mg/100g d.m.]			WATER-SOL.CARBOHYDRATES [%]		
	CONTROL PLANTS	INFECTED PLANTS		CONTROL PLANTS	INFECTED PLANTS	
		STRONG SYMPTOMS	WITHOUT SYMPTOMS		STRONG SYMPTOMS	WITHOUT SYMPTOMS
38	37.56	62.54	13.05	22.16	16.44	18.78
48	31.56	48.75	15.28	13.21	12.47	14.50
23	31.37	44.34	39.12	14.40	10.76	14.82
SK	21.53	24.27	19.88	12.56	15.83	21.16
40	14.14	16.90	6.66	13.84	12.07	17.36
45	11.17	13.51	2.26	16.81	13.82	15.88
22	10.51	27.53	9.97	13.13	11.92	17.21
19	7.16	14.59	2.16	14.16	12.80	18.28
29	6.16	22.91	3.71	14.70	15.78	22.30
47	4.55	13.11	9.06	13.15	12.03	15.48
33	5.70	21.82	2.57	14.66	15.05	18.82
26	4.26	5.61	2.62	15.46	16.57	15.94
52	3.99	1.81	2.30	11.75	18.43	19.25
62	2.90	6.87	2.76	18.85	15.72	21.13
60	2.47	9.34	3.82	18.71	14.95	19.83
56	2.32	5.55	2.32	17.21	16.14	25.30
54	2.27	6.14	3.65	19.24	13.04	13.41
21	1.96	5.35	1.56	11.57	12.22	15.12
36	1.20	1.82	1.78	11.20	14.86	21.48
51	0.91	2.95	1.29	17.30	16.76	15.69
49	0.84	7.71	2.41	17.15	14.92	18.18
AV.	9.74	17.31	7.06	15.30	14.41	18.09

SK = VARIETY SKRZESZOWICKA
AV. = AVERAGE VALUES

RESULTS AND DISCUSSION

Within each genotype there were 3 groups of analysed material:
- infected plants with strong disease symptoms (i.e. the spotting of leaves);
- infected plants without disease symptoms;
- control plants.

The results are shown in Table I. The highest level of perloline occured in plants with strong disease symptoms and ranged from 1.81 to 62.54 mg/100 g d.m. (average 17.31 mg/100 g d.m.). Plants without disease symptoms contained considerably less perloline (from 1.29 to 39.12 mg/100 g d.m., average 7.06 mg/100 g d.m.) than the first group of plants and even less, in most cases, than the third group of plants (from 0.84 to 37.56 mg/100 g d.m., average 9.74 mg/100 g d.m.) (Fig. 1).

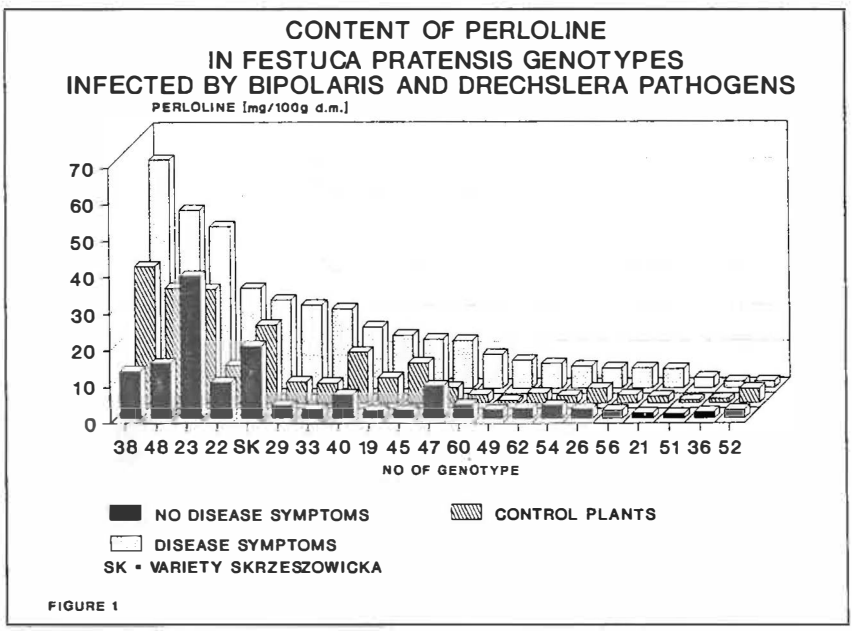


FIGURE 1

The level of water soluble carbohydrates in the plants without disease symptoms was mostly higher than that in the other 2 groups of plants and fluctuated from 13.41 to 25.30% (average 18.09%). The content of water soluble carbohydrates in the plants with

strong disease symptoms ranged from 10.76 to 18.43% (average 14.41%), whereas in the control plants from 11.20 to 22.16% (average 15.30%) (Fig. 2).

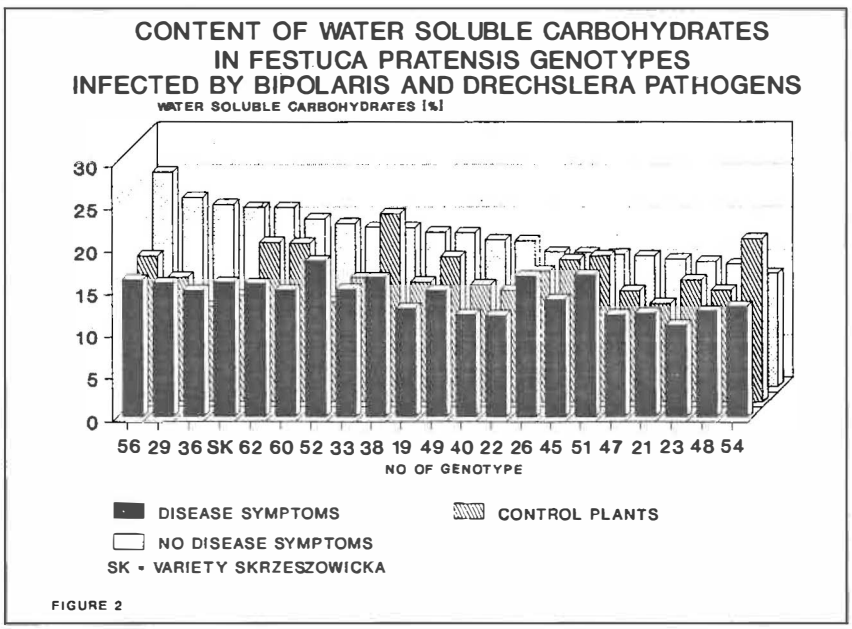


FIGURE 2

The above results indicate that one of the reactions of meadow fescue infected by fungal pathogens is the production of perloline. Some genotypes (nr 38, 48, 23) as well as the Skrzeszowicka variety produce large quantities ofperloline, not only in stress conditions, such as an infection by pathogenic fungi, which is disturbing, considering the fact, that perloline is an antinutritional compound (Aasen et al. 1969; Bush et al.1970; Bush et al. 1972; Culvenor 1973 ; Boling et al. 1975). According to Hegnauer (1963) the level of alkaloids in breeding plants higher than 0.01% of the d.m. could create a real problem as an antiquality factor.

It seems that the significantly low level of perloline in plants without disease symptoms may be connected with the high level of water soluble carbohydrates in this group of plants (Fig. 3). This observation requires further research.

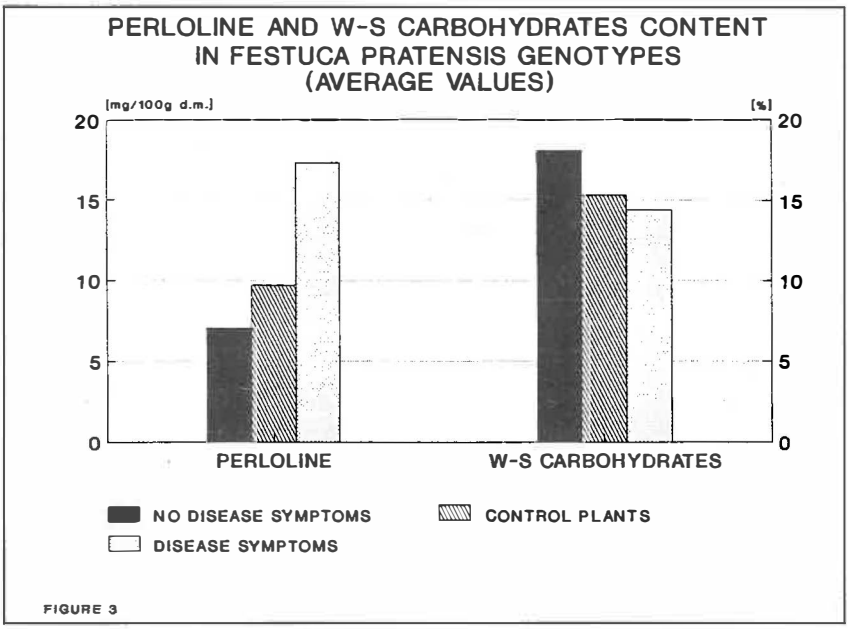


FIGURE 3

CONCLUSION

The differences in perloline concentration were highly significant among the genotypes. At the same time, considering all the genotypes, no pattern emerged between them for the content of perloline and the content ofwater soluble carbohydrates.

However, within the genotype there is a correlation between the content of these compounds for the plants with and without disease symptoms: the plants with disease symptoms contain more perloline and less water soluble carbohydrates in comparison with the plants without disease symptoms.

The content of perloline in meadow fescue, a compound exhibiting activity harmful to animals and probably beneficial for plants, should be an important factor in the quality evaluation of the plant material.

REFERENCES

Aasen A.J.; Culvenor C.C.J.; Finnie E.P.; Kellock A.W.; Smith L.W.,1969: Alkaloids as a possible cause of ryegrass staggers in grazing livestock. Aust. J. Agric. Res., 20: 71-86.

Boling J.A.; Bush L.P.; Buckner R.C.; Pendlum L.C.; Burrus P.B.; Yates S.G.; Rogovin S.P.; Tookey H.L., 1975: Nutrient digestibility and metabolism in lambs fed added perloline. J. Anim. Sci., 40: 972-976.

Bush L. P.; Streeter C.; Buckner R. C., 1970: Perloline inhibition of *in vitro* ruminal cellulose digestion. Crop Sci.,10: 108-109.

Bush L. P.; Boling J. A.; Allen G.; Buckner R. C., 1972: Inhibitory effects of perloline to rumen fermentation *in vitro*. Crop Sci., 12: 277-279.

Culvenor C.C., 1973: Alkaloids., 375-446, In: Butler G.W.; Bailey R.W. (ed), Chemistry and biochemistry of herbage, vol. I, Academic Press, London & New York.

Cunningham I.J.; Clare E.M., 1943: A fluorescent alkaloid in ryegrass (*Lolium perenne* L.) V. Toxicity, photodynamic action, and metabolism of perloline. N.Z.J. Sci & Tech., 24: 167B-178B.

Grimmett R.E. ; Melville J., 1943: A fluorescent alkaloid in ryegrass (*Lolium perenne* L.) I. Introduction. N.Z.J. Sci & Tech., 24: 149B-150B.

Hegnauer R., 1963: The taxonomic significance of alkaloids. In: Swain T., Chemical plant taxonomy, Academic Press, London.

Mendelewski P.; Frencel I., 1985: Perloline occurence in fodder grass green matter of some cultivars and breeding lines of the genera *Lolium* and *Festuca*. Genetica Polonica, 26:3, 307-316.

Reifer I.; Bathurst N.O., 1943: A fluorescent alkaloid in ryegrass (*Loliurn perenne* L.) III. Extraction and properties. N.Z.J. Sci & Tech., 24: 155B-159B

Renner J.E., 1987: Fescue toxicosis, an important intoxication of cattle in South America. Deutsche Tierärztliche Wochenschrift, 94:5, 281-282.

Yates S. G.; Rogovin S. P.; Bush L. P.; Buckner R. C.; Boling J. A., 1975: Isolation of perloline, the yellow alkaloid of tall fescue. I&EC Prod. Res. Dev., 14: 315-319.

Yates S. G.; Fenster J. C.; Bartelt R. J, 1989 : Assay of tall fescue seeds extracts, fractions and alkaloids using the large milkweed bug. J. Agr. Food Chem., 37:2, 354-357.

Structural diversity of Palmarumycins

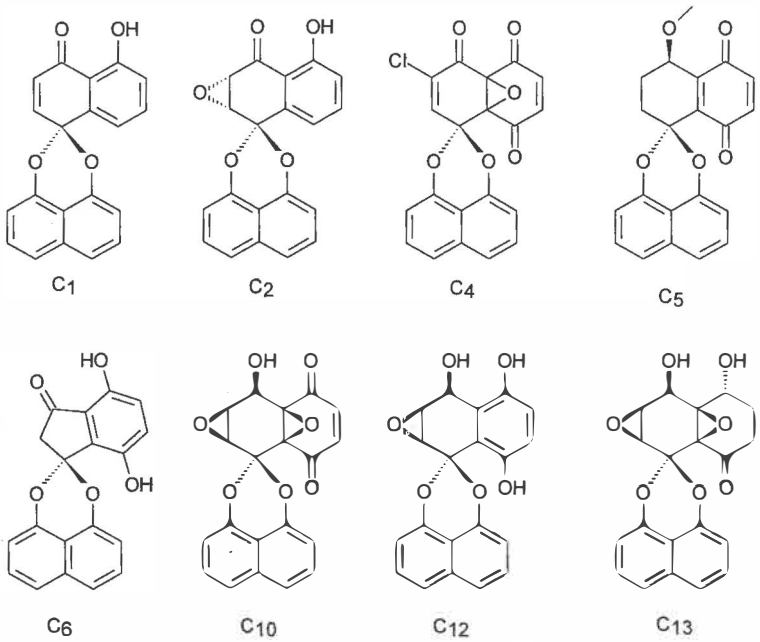
K. Krohn[a)], A. Michel[a)], K. Beckmann[a)], H.-J. Aust[b)], B. Schulz[b)], S. Draeger[b)]

a) Universität-Gesamthochschule Paderborn, Fachbereich Chemie und Chemietechnik
b) Institut für Mikrobiologie der TU Braunschweig.

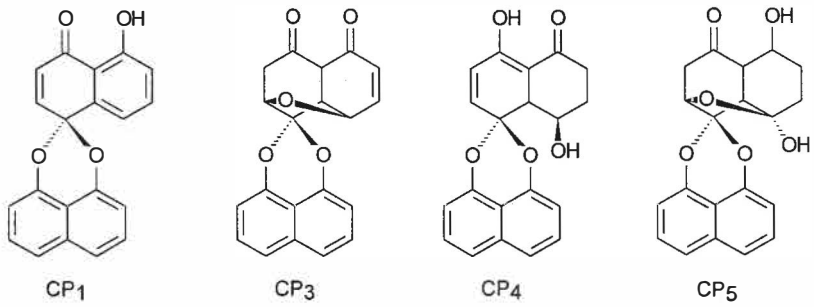
Introduction

In connection with a screening program for biologically active compounds with potential for use in crop protection, we have investigated a number of endophytic fungi. The structurally new class of the palmarumycines was isolated from the fungus *Coniothyrium* sp. and *Coniothryium palmarum* . The compounds vary in structure and biological activity. In most cases these particularly interesting compounds showed a spiro-acetal connection between two naphthaline derived fragments, but exceptions are also known. Altogether we isolated twenty-five palmarumycines and determined their structures [1-4]. With the following examples we want to show the great structural diversity of this interesting new class of natural products.

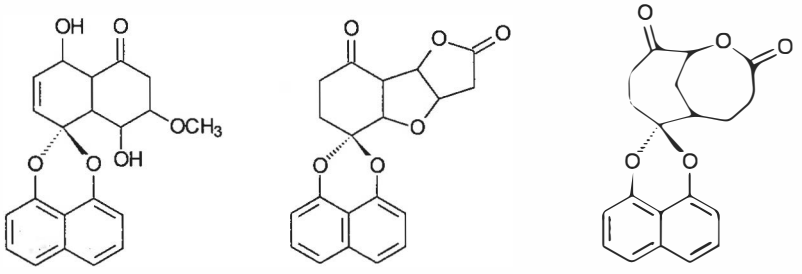
Structures



Metabolites produced by an unindetified *Coniothyrium* species isolated from forest soil in West Borneo.



CP1 CP3 CP4 CP5

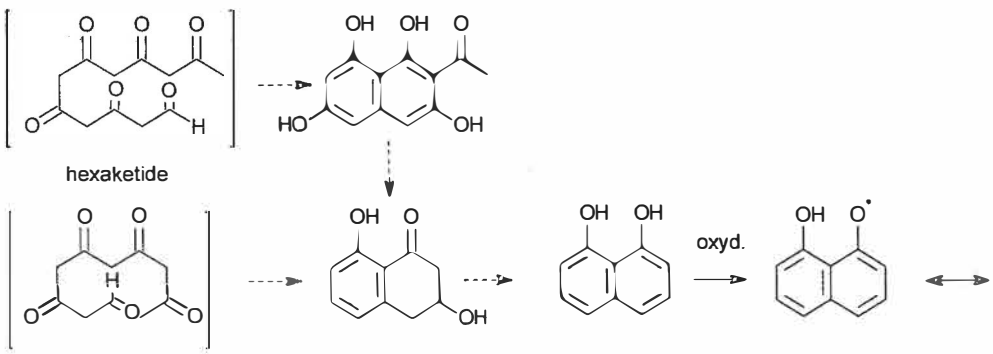


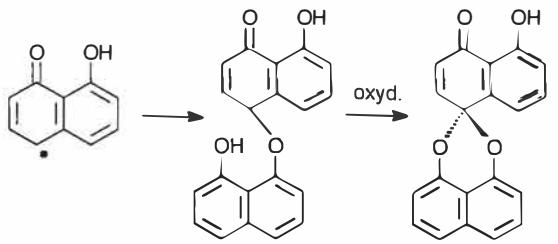
CP6 CP7 CP8

Metabolites produced by the endophyt *Coniothyrium palmarum* isolated from *Lamium purpureum*.

Biosynthesis

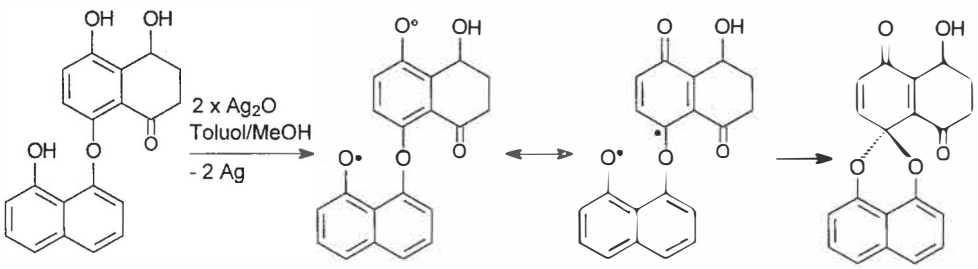
Hypothetical Biosynthesis of Palmarumycin CP 1





Semipreparative Synthesis of a new Palmarumycin

Starting from the novel secondary metabolite 4,5-dihydroxy-8-(8-hydroxynaphthalene-1-yloxy)-3,4-dihydro-2H-naphthalene-1-one, recently isolated from *Coniothyrium palmarum*, the synthesis of a new palmarumycin was achieved. This semipreparative synthesis is also a confirmation of our hyphothetical biosynthesis[4].



Reaction conditions: 60 h at 20 °C. Yield: 68 %.

Biological Activity:

The biological activity was determined as inhibition of test organisms in agar diffusion tests and inhibition of the germination and growth of garden cress. In these tests the antifungal activity was most pronounced.

Radius of inhibition [mm]								
Comp.	Chl.	E.c.	B.m.	Ust.	Eur.	M.m.	Fus.	Cress Inhib. [%]
C_2	3	1	3	7	5	1	3	0
C_4	0	6	8-10	10-12	15-17	2	5	100
C_6	0	0	0	6	5	-	0	0
C_{10}	0	6	8-10	10-12	15-17	2	5	-
C_{12}	1	4	5-7	10	15	2	5	30
CP_1	0	2	3-4	5-7	7-10	0	0	-
CP_2	3	0	0	0	2	0	0	-
CP_3	2	5-8	8	20	28-30	26	15	-
CP_4	6	5	6-8	8-10	9-10	4	7	-
CP_6	2	0	5	9-10	4-6	6	3	-
CP_7	1	0	0	1	1	0	0	-

Test organisms:

Chl. *Chlorella fusca*
E.c. *Escherichia coli*
B.m. *Bacillus megaterium*
Ust. *Ustilago violacea*
Eur. *Eurotium repens*
M.m. *Mycotypha microspora*
Fus. *Fusarium oxysporum*
- not tested

Literature

[1] A. Michel, *Isolierung, Strukturaufklärung und Syntheseversuche biologisch aktiver Sekundärmetabolite aus Coelo- und Hyphomyceten*, Dissertation, Paderborn **1994**.

[2] K.Krohn, A. Michel, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, *Liebigs Ann. Chem.* **1994**, S 1093-1097.

[3] K.Krohn, A. Michel, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, *Liebigs Ann. Chem.* **1994**, S 1099-1108.

[4] K.Krohn, K. Beckmann, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, *Liebigs. Ann. Chem.* **1996.**, In preparation.

Product Quality

Product quality - its significance in grass production

Johannes Isselstein

Institut für Pflanzenbau und Pflanzenzüchtung II, Grünlandwirtschaft und Futterbau, Justus-Liebig-Universität Gießen, Ludwigstr. 23, D-35390 Giessen

ABSTRACT

Grass production has paid increasing attention to product quality over the last years. This concerns the herbage production for livestock as well as the production of turf and lawn grasses. General quality aspects of grassland products are discussed in the paper. In future a high product quality has to be achieved in a sustainable and environmentally sound production system. Therefore in a wider perception of quality it should be considered whether the production process is in agreement with environmental concerns. It is a challenge for farmers and scientists to develop grassland systems that ensure a high product quality produced under low input conditions.

INTRODUCTION

Grassland provides a wide range of products. Producing fodder for livestock used to be the only purpose of grass production over decades. In recent years however, other purposes of grassland have come to the fore and for West and Central Europe the supply of forages is no longer playing the dominating role. Other goals of grass production are the creation of turfs and lawns for leisure activities and recreation. In addition with any kind of grassland management environmentally significant by-products are being produced, e. g. the contribution of grassland to the biodiversity of a landscape or the ground water share. Those aspects have received increasing attention during the last years. According to the broadened perception of grass production the comprehension of product quality has become more complex.

In general the quality of an agricultural product is characterized by its internal and external features and its utility value, i. e. it depends on the usage. Main criteria are

In general the quality of an agricultural product is characterized by its internal and external features and its utility value, i. e. it depends on the usage. Main criteria are therefore the constitution or make-up, e. g. the size, the taste, the colour or the smell of a product. The nutritive value is of course of outstanding significance for food. Product quality often comprises the suitability of food or fodder for further processing, e. g. making chips from potatoes or silage from grass. These characteristics reflect the physical, chemical and biological constitution of the product itself. The production process is usually not seen as part of the quality of the product being produced with it. However, for farmers and scientists it seems worth to consider those aspects of quality as they are already taken into account by consumers in their shopping behaviour. A good of a high quality should have been produced in a sustainable and environmentally friendly way.

FORAGE VALUE FOR RUMINANT FEEDING

The aim of grass production for ruminants is to produce milk and meat. A high quality grass therefore ensures an efficient turnover of plant nutrients into animal product. Looking at it more detailed the quality is determined by the concentration of valuable contents like proteins, carbohydrates, minerals etc. The utilization of the plant nutrients by the animal, the digestibility and palatability, is of paramount importance. In addition there are other characteristics affecting the quality of grass like the conservation properties for hay-making or ensilage, the persistence and competitive strength in mixed swards, the cold resistance as well as the pest and disease tolerance or resistance. Regarding the production process grass should be produced in a system that does not pollute the environment and that maintains the biodiversity. Organic grass farmers e. g. restrict themselves in the use of external inputs like mineral fertilizers and concentrate feed stuff, thus they provide a special image with their products, some sort of non-material value. In many industrial countries this idea is accepted by consumers as they are willing to pay a higher price for the product.

With the introduction of the milk quota system in the European Community and the lowering of product prices in recent years the intensity of grassland utilization has generally decreased. As a consequence farmers try to increase the efficiency of grass production by minimizing costs. Means to reduce costs are the limited use of agrochemicals in particular nitrogen fertilizer and no purchase of concentrate feed stuff.

The consequences of such restrictions regarding animal feeding have to be considered. In principle animal performance is strongly dependent on the forage value. An example for this is shown in Figure 1.

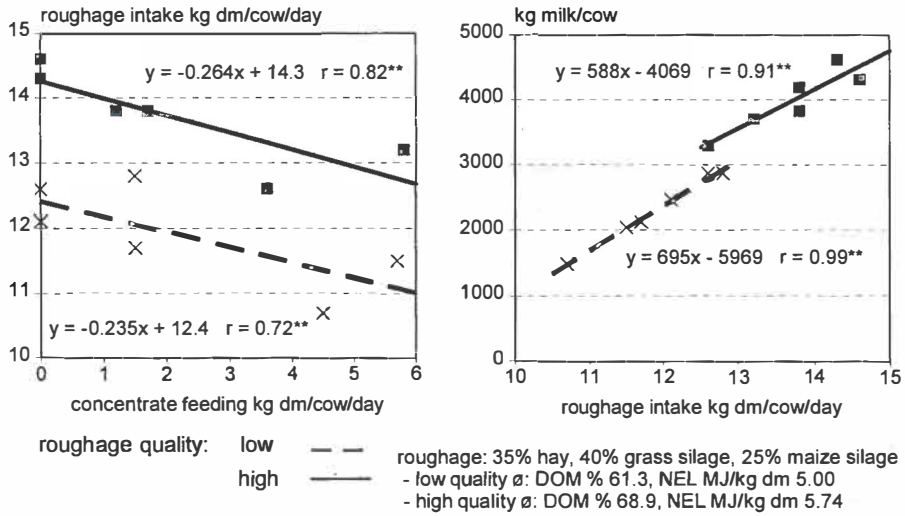


Figure 1: Roughage intake and milk production from low versus high quality roughage (Gruber et al. 1995)

In an indoor trial 120 dairy cows were fed two different roughages throughout a whole lactation period. The roughage consisted of 35 % hay, 40 % grass silage and 25 % maize silage. Organic matter digestibility (DOM) and net energy lactation value (NEL) were either 61.3 % and 5.00 MJ kg^{-1} dry matter for the low quality or 69 % and 5.74 MJ for the high quality, respectively. The breeds tested were Holstein Friesian and Simmental. Due to no treatment x breed interactions the data are averaged. As expected the daily roughage intake decreased with increasing concentrate feeding. Forage intake of the good roughage quality was significantly higher compared to the low quality. In terms of annual milk yield the high quality roughage was markedly superior to the low quality and performance losses as a result of low roughage quality could not be compensated by increased concentrate feeding. The annual milk yield without any concentrates was 4.5 t per cow for the high and 2.6 t for the low quality. It can be concluded that the better the grass quality the better the chances to do without concentrate feeding. However, the

question has to be answered whether a high grass quality is attainable in a low input system operating without or with reduced mineral nitrogen fertilizer. Low input management is often accompanied by an increase of not sown species in the sward like forbs and secondary grasses. This obviously has an impact on the forage quality as is demonstrated by an experiment performed in Scotland (Figure 2).

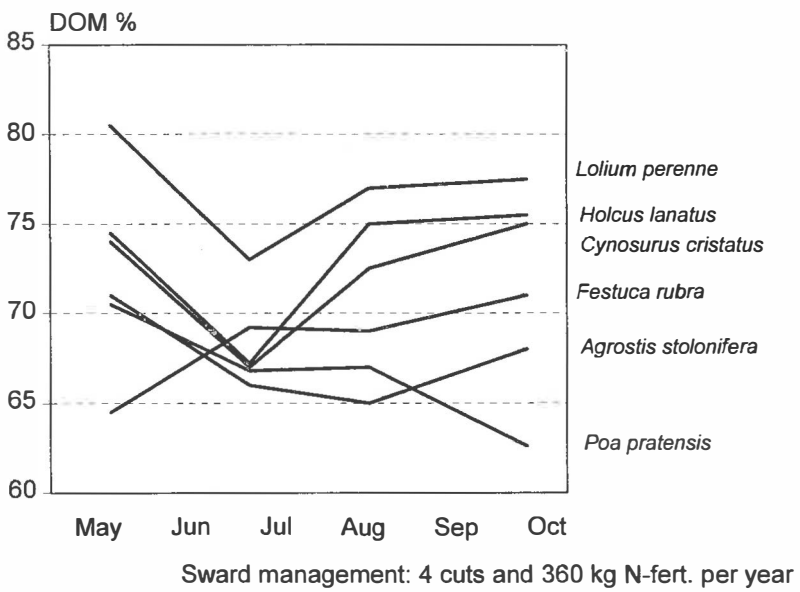


Figure 2: Digestibility of different grass species cut for silage (Frame 1989)

The digestibility of secondary grasses in a silage cut system was compared to that of *Lolium perenne* and *Poa pratensis*. Data are averaged over three successive years. *L. perenne* displayed the highest digestibility throughout the growing season. All other species were of minor value. In West and Central European conditions there is only one species - *Trifolium repens* - showing a high quality comparable to *L. perenne*. Therefore it seems that plant breeding and management effort in developing low input systems for dairy cattle should focus on the competitiveness and persistence of *L. perenne* and *T. repens* rather than on the improvement of the quality of secondary grasses. This concerns in particular the winter hardiness of *L. perenne* which is often a problem in Central Europe as well as the cold tolerance of *T. repens*. In addition drought tolerance and disease resistance of both species should be improved. Apart from these aspects of grass quality

the susceptibility to weed encroachment is another characteristic for the quality of a grass. For instance, as long as the competitiveness and productivity of *L. perenne* can be maintained the risk of serious weed infestation is low. The effect of various management factors on the weed encroachment into ryegrass stands was examined in a field trial performed at Giessen. Grass swards of two ryegrass varieties - Liprior, an early flowering cutting type, and Barezane, a late flowering pasture type - were established and either lowly or highly fertilized. One year after sward establishment freshly ripened diaspores of *Taraxacum officinale* and *Plantago lanceolata* were broadcast over the grass sward; The fate of emerging seedlings was continuously followed. Emergence and establishment of the forbs was high and took place in a relatively short time. In terms of dry matter yield *P. lanceolata* almost outcompeted *L. perenne* within a period of two years (Figure 3). Weed establishment was affected by N-fertilization and ryegrass variety. N-fertilization markedly increased the competitive strength of *L. perenne* and the establishing success of the forbs was reduced. There was a tendency that the cultivar Liprior was slightly superior to Barezane in controlling (competing with) the weeds. This result demonstrates that the performance and persistence of ryegrass depends on a sufficient availability of nitrogen. In a low-input system this can be achieved by introducing white clover as a companion species. Chances seem to exist for the selection of suitable, i. e. competitive ryegrass varieties which provide a higher persistency of the grass sward.

Concerning the environmental impact of grass production a balanced nutrient turnover on the farm level appears to be an adequate measure for the compatibility of production and environmental interests. Reducing purchase concentrate feed and N-fertilization are effective means to decrease a nutrient surplus and are therefore considered as an quality improvement of the production process.

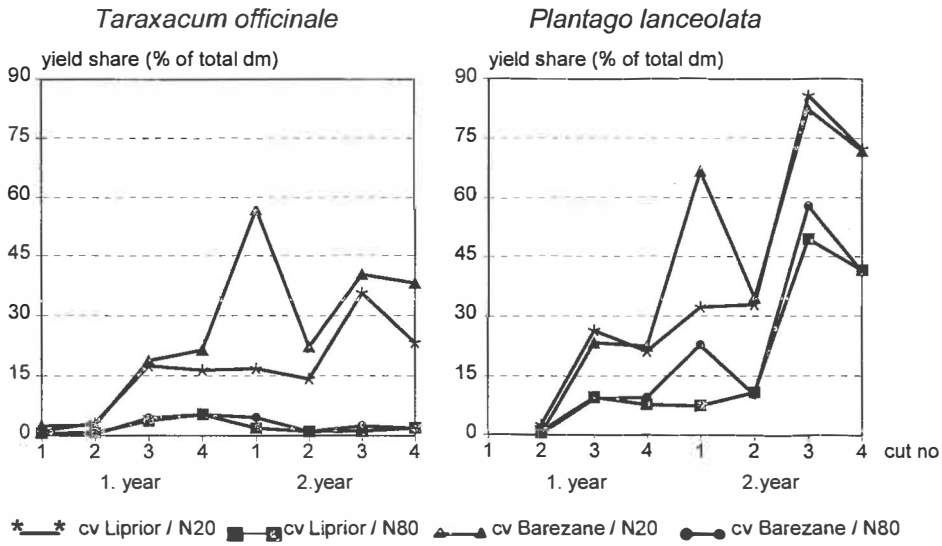


Figure 3: Effect of ryegrass variety (*Lolium perenne*) and N-fertilization on the establishment of *Taraxacum officinale* and *Plantago lanceolata* (Hofmann et al. 1996)

QUALITY OF TURF AND LAWN GRASSES

The quality characteristics of turf grasses are variable according the usage. Some quality characteristics are however advantageous in any case. A rapid seedling emergence facilitates a dense plant cover within a short time which reduces erosion risks of reseeded turfs. Also disease and pest tolerance or resistance is of outstanding importance for a high quality grass of all kind of turf. Other criteria of turf and lawn grasses are whether they have the potential to build a dense and competitive sward with a low susceptibility to weed invasion. Garden lawns should have a strong green colour. Landscape lawns on the other hand should provide some aesthetic and/or nature conservation value, i. e. a high biodiversity and a broad range of flowering species of different colour. Turf grasses should develop a resilient turf which is resistant to wear and tear. Therefore a good regrowth potential is required. Similar to grasses grown for agricultural purposes the management of turfs and lawns has to be compatible with environmental interests. A high turf quality with respect to technical and aesthetic values seems no longer to be acceptable if it comes along with an increased risk of environmental pollution. However, are there options to minimize pollution risks from

intensively managed turfs? In practice such turfs often are exposed to high doses of nitrogen fertilizer and consequently exhibit a considerable nitrate leaching potential. In an experiment at Giessen it was investigated whether the botanical composition of an intensively managed turf has an impact on the nitrate accumulation in the soil (Figure 4).

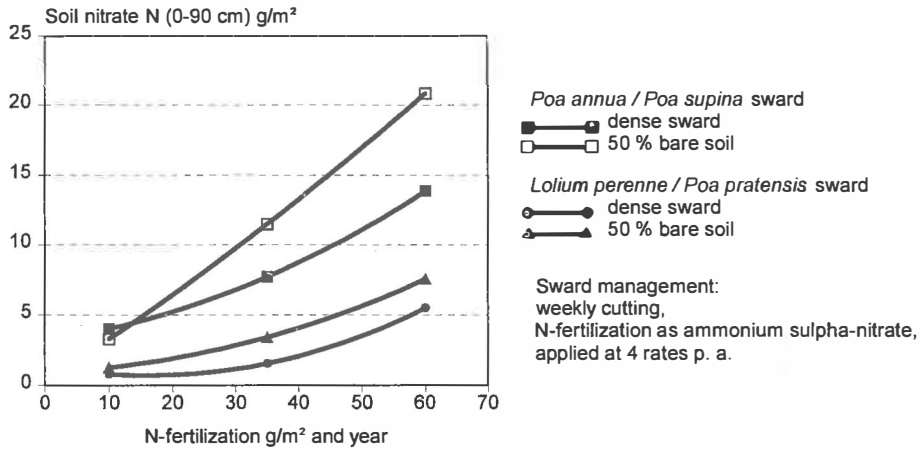


Figure 4: Soil nitrate accumulation at the end of the season of different grass swards and varying N-fertilization (Opitz von Boberfeld 1995)

Two different swards were tested, the one was dominated by *Poa annua/Poa supina*, the other by *L. perenne/Poa pratensis*. The sward density was varied, i. e. it was either dense or the vegetation was partially removed leaving 50 % bare ground. The latter should simulate a heavily trampled turf like the penalty area of a football ground. The swards were cut weekly, N-fertilizer was applied as ammonium sulpha-nitrate at four rates per annum. Increasing fertilizer applications led to an increased soil nitrate accumulation at the end of the season. In a winter humid climate this nitrate is likely to be leached into deeper soil layers and consequently into the ground water. The soil nitrate content was markedly higher under the *P. annua/P. supina* sward compared to the *L. perenne/P. pratensis* sward. This difference further increased when the plant cover was reduced to 50 %. Because of a more extensive rooting system *L. perenne* and *P. pratensis* could make better use of nitrate mineralized in gaps of the sward and therewith reduce the leaching potential. This example demonstrates the necessity to select adequate species and genotypes to control pollution risks. The selection of species is also important for turfs

aiming at the minimization of cultural practices like fertilization, irrigation, pesticide use, mowing, etc. which are required to maintain the sward. This is demonstrated by an experiment with different *Festuca* species grown without fertilization and irrigation.

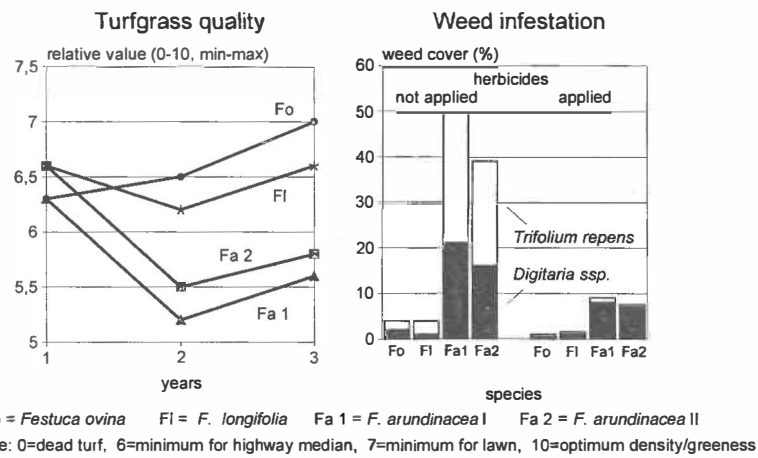


Figure 5: Turf grass performance without fertilization and irrigation (Dernoeden et al. 1994)

The herbicide application was varied. The visually determined turf quality (turf cover, colour, density) was satisfactory and even increased with time for *F. ovina* and *F. longifolia* (Figure 5). Weed invasion was low even though herbicides were not sprayed. *F. arundinacea* on the other hand was less suitable for a low-maintenance turf. The turf quality was significantly lower and the application of herbicides necessary. Both examples, the intensively managed sward as well as the low-maintenance turf demonstrate that with the choice of adequate species an environmentally sound management is generally possible. If produced in such a way the production process should be emphasized as a special quality characteristic of the product.

CONCLUSIONS

The product quality has received increasing attention in recent years in grass production due to changing economical framework as well as consumer demands. This development is likely to maintain and even increase in future. A broader comprehension

of product quality that includes the production process as part of the quality will further intensify this development. Trying to sell their products the producers be asked to disclose their way of production whether it is compatible with environmental and social concerns. This is not only a challenge for the producers but also for scientists to develop production systems that are in agreement with those concerns.

REFERENCES

Dernoeden, P.H., Carroll, M.J., Krouse, J.M., 1994: Mowing of three Fescue species for low-maintenance turf sites. Crop Science 34, 1645-1649.

Frame, J., 1989: Herbage productivity of a range of grass species under a silage cutting regime with high fertilizer nitrogen application. Grass and Forage Science 44, 267-276.

Gruber, L., Steinwender, R., Baumgartner, W., 1995: Einfluß von Grundfutterqualität und Kraftfutterniveau auf Leistung, Stoffwechsel und Wirtschaftlichkeit von Kühen der Rasse Fleckvieh und Holstein-Friesan. Bericht über die 22. Tierzuchtagung, Aktuelle Forschungsergebnisse und Versorgungsempfehlungen in der Rindermast und Milchviehfütterung, BAL Gumpenstein, Austria, 49 p.

Hofmann, M., Isselstein, J., Opitz von Boberfeld, W., 1996: Entwicklung in *Lolium perenne*-Grasnarben eingesäter Kräuter und Bedeutung für die Ertragsleistung der Bestände. Pflanzenbauwissenschaften, (submitted).

Opitz von Boberfeld, W., 1995: Steuerung des Nitratgehalts von Böden. Spiegel der Forschung 12 (2), 26-27.

The incidence and significance of brown rust (*Puccinia recondita* f.sp. *lolii*) and stem rust (*Puccinia graminis*) in herbage seed crops in the UK

J E THOMAS AND A J CAMPS

National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 OLE UK

ABSTRACT

Seed production plots of perennial and Italian ryegrass in the UK were affected by two diseases, brown and stem rust, which are seldom seen at high levels in grass grown for conservation or grazing. There were significant differences between cultivars in resistance to the rusts, and these are discussed in relation to disease control strategies. Preliminary investigations on the epidemiology of stem rust have been carried out in order to determine likely high disease risk seasons

Key words: ryegrass seed crops; *Puccinia recondita* f.sp. *lolii*, *Puccinia graminis*

INTRODUCTION

Herbage seed crops are grown on about 10,000 ha annually in the UK, predominantly in the east and south of the country. Perennial ryegrass comprises about 8000 ha, and Italian ryegrass about 1500 ha. Other species (cocksfoot, fescues and timothy) are grown on a total of about 1000 ha (Nix, 1995). Foliar fungal diseases are thought to reduce seed yield in ryegrasses, and these species have received most attention with respect to disease control measures (Hampton and Hebblethwaite, 1984). Most crops are treated with at least one fungicide spray during the season, but in trials the increases in seed yield associated with fungicide application are variable (Poole, 1992), and commercial sprays may not always be cost effective. The range and significance of diseases appearing in seed crops has not been investigated fully, and there is consequently relatively little information on the disease resistance of cultivars when these are grown as seed crops.

information on the disease resistance of cultivars when these are grown as seed crops. Variable responses to fungicide may be associated with poor timing of application in relation to the development of specific diseases, and a lack of knowledge of cultivar resistances. This work was undertaken to identify the most common and severe diseases in ryegrass seed crops in the UK, and to assess the resistance of a range of commercial cultivars to those diseases. Initial experiments were also carried out to investigate the conditions which favour the development of stem rust, one of the pathogens identified. The results are discussed in relation to improving the effectiveness of fungicide applications.

MATERIALS AND METHODS

a) Field trials

Plots of a range of perennial and Italian ryegrass cultivars which are grown for seed commercially in the UK were established at two sites in the east and south of the country (National Institute of Agricultural Botany trial centres in Cambridge and Hampshire respectively) which are representative of the major seed growing area. Two replicate plots, 5m x1m of each variety were sown at each site, with seed rates equivalent to 12 and 18 kg/ha for perennial diploid and tetraploid types, 18 kg/ha for Italian diploids, and 30kg/ha for Italian tetraploids or hybrid tetraploids. The plots were maintained according to local agronomic practice for seed crops with the exception of fungicide application. At the site in the south, Italian ryegrass plots were cut to a height of 6cm at the end of March to reflect the grazing management applied to some seed crops, but plots were left uncut at the eastern site. All plots were recorded for one season only, and then removed. Separate trials were sown in 1992, 1993 and 1994, with some common cultivars being included each year, and recorded in 1993,1994 and 1995. Plots were inspected from the end of March until the end of July, and foliar fungal disease recorded for each plot as the % of leaf or stem area infected (LAI or SAI)

b) seedling tests

Seedlings of cv. Aubisque (susceptible to stem rust) were inoculated at the three leaf stage with a mixture (1/20, v/v) of uredospores of an isolate of *P. graminis* (collected at Cambridge in 1994) and talc. Plants were placed in plastic bags containing a small amount of water at either 7 or 20 °C for 48h in the dark, then removed and placed in one

of the following environments: a growth cabinet at continual 15 °C with 18h light provided by "Warmwhite" fluorescent tubes, a growth cabinet at continual 25° C, also with 18h light, and a glasshouse at 20 °C (day) and 18 °C (night), with natural daylight in June. There were 120 seedlings per treatment, with 60 in each of two replicate trays. Plants were monitored for the appearance of pustules from 6 days after inoculation, and severity of disease was recorded as % leaf area infected for each plant, and a mean for 60 plants calculated.

RESULTS

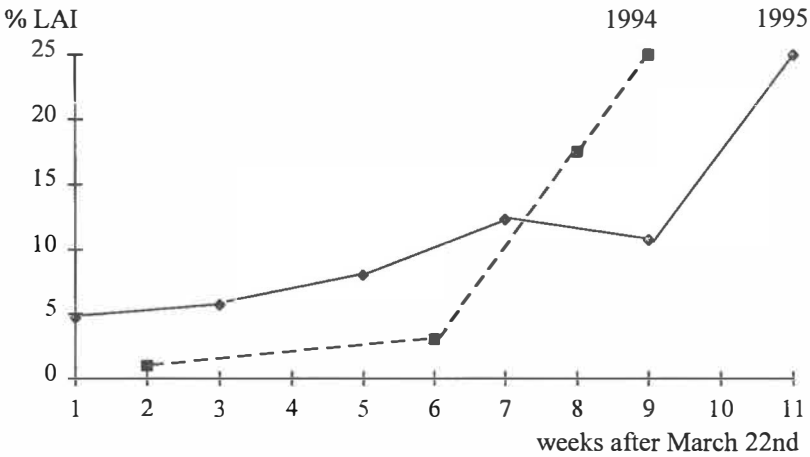
a) Field trials

In one year out of the three, leaf blotch (*Rhynchosporium orthosporum*) exceeded 5% LAI on some cultivars in the south. Leaf spots (*Drechslera siccans* and *D. andersenii*) were recorded at low levels (between 0 and 5%) at both sites every year. However, those diseases which exceeded 5% LAI or SAI every year at each site were mildew (*Erysiphe graminis*), brown rust (*Puccinia recondita* f.sp. *lolii*) and and stem rust (*Puccinia graminis*). Overall, levels of mildew were higher in the east than in the south. However, levels of both rusts were generally higher than those of mildew on the most susceptible cultivars for each disease in the trials. Dates of first appearance of brown and stem rusts are given in Table 1. Progress of both diseases was monitored in detail at the eastern site in 1994 and 1995 and progress curves on the most susceptible cultivars are shown in Figure 1a and 1b. Epidemics of brown rust were halted by leaf senescence around the end of June on perennial ryegrass, and about 14 days earlier on Italian ryegrass. Stem rust increased rapidly at the beginning of July, particularly in 1995, and was halted by whole crop senescence at the end of the month. Italian ryegrass crops matured about 14 days earlier than most perennials, and only very low levels of stem rust were seen on this crop. Certain very early flowering cultivars (cvs Cherokee and Numan) of perennial ryegrass also had only low levels of stem rust, but these cultivars had senesced at the time when stem rust was increasing on the green stems and florets of other cultivars.

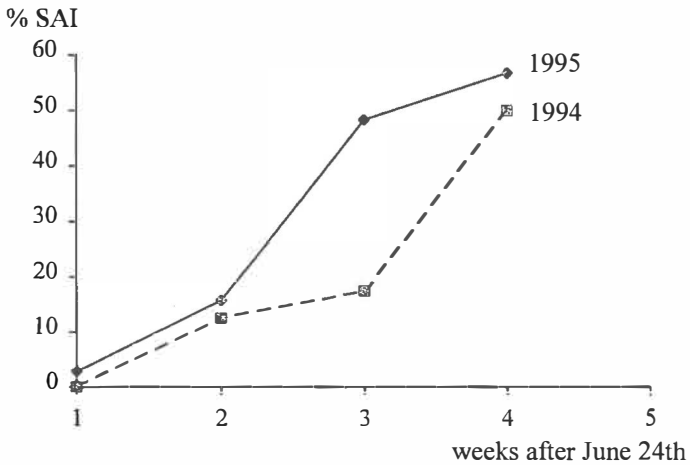
The range of resistance to brown and stem rusts is summarised in Table 2. Significant differences between severity of disease occurred for both rusts, but the level of resistance available in the range of material tested was generally higher for brown rust than stem rust.

Figure 1

a) Disease progress of brown rust (east) in 1994 and 1995 on Italian ryegrass cv Abercomo (1994) and perennial ryegrass cv Twystar (1995)



b) Disease progress of stem rust (east) in 1994 and 1995 on perennial ryegrass cv Fennema (1994) and Complex (1995)



Of nine Italian ryegrass cultivars tested in 1995, three had levels of susceptibility similar to that of cv Abercomo, of the remainder, two were as resistant as cv Solid. Of a total of 29 perennials tested over all maturity groups, eight were as susceptible as cv Labrador, and seven as resistant as cv Complex. For stem rust, 12 were similar to cv Complex or Portstewart, and eight were as resistant as cvs. Abersilo or Choice. There was no apparent differences in the distribution of resistant or susceptible cultivars within maturity groups, and though tetraploid Italian cultivars were generally resistant to brown rust, some were as susceptible as diploids. Several perennial cultivars (eg cv Choice) were highly resistant to one rust and susceptible to the other.

Table 1 Dates of first appearance of brown rust and stem rust at two sites from 1993-1995

Year	South		East	
	Brown rust	Stem rust	Brown rust	Stem rust
1993	22 June	8 July	15April	4July
1994	16 May	7 July	20 April	5 July
1995	16 May	17 July	22 March	4 July

Table 2 Severity (% leaf area infected) of brown rust on cultivars of Italian and perennial ryegrass (examples from 1995 data only)

	Italian ryegrass	Perennial ryegrass	
		Early and intermediate	Late
Maximum	25.0 (cv Abercomo)	23.0 (cv Labrador)	25.0 (cv Twystar)
Minimum	5.7 (cv Solid)	3.0 (cv Complex)	2.3 (cv Tivoli)
lsd (p=0.05) for cv means	3.15	8.72	7.27

Table 3 Severity of stem rust (% stem area infected) on cultivars of perennial ryegrass (examples from 1995 data only)

	Perennial ryegrass	
	Early and intermediate	Late
Maximum	56.7 (cv Complex)	50.0 (cv Portstewart)
Minimum	25.0 (cv Abersilo)	33.0 (cv Choice)
lsd (p=0.05) cv means	6.93	6.87

b) Seedling tests

Results from seedling tests indicated that the locally collected isolate of stem rust developed most rapidly under a continually high temperature, but that the initial temperature during the 48h period of high humidity had relatively little effect on the time of appearance of pustules, or the severity of disease (Table 4).

Table 4 The incidence and severity of stem rust pustules on leaves of cv Aubisque after incubation under various temperature regimes 8 and 14 days after inoculation (DAI) respectively

	No. of leaves with pustules 8 DAI			Mean severity (% LAI) 14 DAI		
48h dew	15° C	20 °C	25° C	15° C	20° C	25 °C
7 °C	0	0	39	0.5	7.8	11.6
20 ° C	0	3	43	0.5	12.2	11.2

DISCUSSION

The incidence of an early and late season rust disease on ryegrass seed crops, and the existence of a large range of cultivar resistance to each disease, suggests that a revision of the advice concerning timing of fungicide applications is required. For those cultivars which are particularly susceptible to brown rust, an early season spray during tillering around mid-April may be necessary to prevent the rust reaching high levels. This may be appropriate for some Italian ryegrass cultivars which are not grazed in late March, and which therefore may harbour high levels of inoculum, and a few perennial cultivars. For most however, it seems likely that the usual mid May timing at flag leaf emergence would be sufficient to prevent an increase of the disease in June. It is debatable whether this timing will give sufficient protection under all circumstances against the late season stem rust, which starts to develop some six weeks later. Cultivars which are moderately or highly susceptible to stem rust could benefit from an additional spray at around the end of June, or during anthesis.

Neither brown or stem rust are common in grass grown for conservation or grazing, though levels of brown rust can increase in some Italian ryegrass silage crops (Anon, 1995), and stem rust has occasionally been noted on grass during the late summer and autumn in the UK (Varney *et al.,* 1992). Growers are largely unaware of these diseases, and of the range of cultivar resistance available, and it seems likely that at least some of the variable responses to fungicide seen in trials with different cultivars have been due to the different levels of resistance to brown and stem rust.

The rapid and comparatively early increase of stem rust in 1995 may be due to the very high temperatures experienced that year. Mean maximum July temperature was 25.8°C; 4 °C above the 30 year mean and the 1994 mean. Results from the seedling test indicated that the development of a locally collected isolate of stem rust was more rapid at temperatures exceeding 20°C. Interestingly, however, the low temperature during an initial period of high humidity did not greatly decrease the development of disease at a subsequent high incubation temperature, and it is possible that this stem rust may become established at relatively low temperatures, but that further rapid growth in the leaf and sporulation is encouraged by much higher temperatures. If so, this may explain the sudden and extremely rapid development of disease which occurred over a 7 day period in 1995.

It is not known whether the isolate of stem rust infecting ryegrasses in the trial areas belongs to one of the recognised *forma speciales*. Roderick *et al.* (1994) considered that the stem rust seen on ryegrass in Wales probably belonged to *P. graminis* ssp *graminicola*. Limited host range tests with the isolate from eastern England (Thomas, unpublished results) demonstrated sporulation on barley, but not wheat, oats or red fescue. Further information on graminaceous hosts, and possible alternate hosts, together with more detailed epidemiological studies, may enable prediction of seasons when stem rust is likely to be severe. Brown rust has been described as *f.sp. lolii* (Williams, 1984), and extensive host range tests on graminaceous species indicated that sporulation only occured on *Lolium perenne*, *L. multiflorum* and *Festuca pratensis* (Wilkins, 1973). The disease has been observed at particularly high levels following mild winters, and it is possible that such conditions permit the survival of the fungus as the uredial stage on ryegrass. Defoliation of swards for forage takes usually takes place in early May, and thus it is predominantly seed crops where the disease may continue to develope.

REFERENCES

Anon (1995) Recommended Varieties of Grasses and Herbage Legumes, National Institute of Agricultural Botany, Cambridge.

Hampton, J.G. and Hebblethwaite P.D. (1984) The effect of fungicide application on seed yield in perennial ryegrasscv S24. Annals of Applied Biology, **104** 231-239.

Nix, J. (1995) Wye College Farm Management Pocketbook, 1995, Wye College.

Poole, N. (1992) "High Herbage Hopes", in "Crops" magazine, **10** no 9, p37, Reed Business Publishinhg, Surrey, UK

Roderick, H.W., Clifford, B.C. and Jones, J.E. (1994) Occurrence of oat stem rust in west Wales. Plant Pathology **43**, 223-224.

Varney, P.L., Wedgwood, E.F., Thomas J.E. and Broom, E.W. (1992) Incidence of stem rust (*Puccinia graminis*) on perennial ryegrass cultivars. Tests of Agrochemicals and Cultivars No 13, Ann Appl Biol **120** (Supplement), 118-119.

Wilkins, P.W. (1973) *Puccinia recondita* on ryegrass. Plant Pathology **22**, 198.

Williams, R.D. (1984) Crop protection Handbook-Grass and Clover Swards, Chapter 7, p54. BCPC Publications, Croydon, England

ACKNOWLEDGMENT

This work was supported by a grant from the National Farmers Union, UK

Impact of selection for *Xanthomonas* resistance on yielding ability of Italian ryegrass in Switzerland

BOLLER, B.; LEHMANN, J.

Eidgenössische Forschungsanstalt für Agrarökologie und Landbau, FAL Reckenholz, CH-8046 Zürich, SWITZERLAND

ABSTRACT

We investigated the relationship between resistance to *Xanthomonas* and dry mass yield of 42 Italian ryegrass (*Lolium multiflorum ssp. italicum*) varieties tested in the recommended list trials for Switzerland. A single visual score (1 to 9) of the disease was given when it became first apparent at two locations in June (during the second growth) of the first year after the year of sowing. There was a strong relationship between this score and dry mass (DM) yield during the rest of the growing season. Annual DM yield, averaged over both locations, declined by 4 % for each unit of the *Xanthomonas* score. For both locations, the steepest regression line for an individual cut (-11 % DM yield for each unit of the disease score) was observed for the third growth. There was only a weak correlation between the *Xanthomonas* score and yield of the first growth, indicating that yielding ability of even the most susceptible varieties was practically intact prior to the first record of the disease. A parallel experiment with a choice of Italian ryegrass varieties grown either as pure stands or in a mixture with red clover (*Trifolium pratense*) showed that the more susceptible varieties were outcompeted to a higher degree by the companion clover when affected by the disease.

Keywords: *bacterial wilt, Lolium multiflorum, cultivars*

INTRODUCTION

Bacterial wilt first became apparent as an important grass disease about 1970 when unfamiliar wilt symptoms were observed in the Italian ryegrass breeding nursery of our

research station. The pathogen was identified as belonging to the genus *Xanthomonas* and described by Egli *et al.* (1975). The strong natural infection pressure obviously contributed to a moderate resistance of the cultivars released in that period, namely LIPO and TURILO. Since 1977, an artificial infection method, developed by Schmidt (1976), is being used systematically for improving *Xanthomonas* resistance of ryegrass at Zürich-Reckenholz.

In Switzerland, ryegrass stands are regularly attacked by *Xanthomonas*. Resistance against the bacterial wilt disease is therefore a prerequisite for obtaining satisfactory yields from Italian ryegrass cultivars. We investigated the relationship between *Xanthomonas* resistance and yielding ability by analyzing the results of the 1992 to 1994 recommended list trials for Switzerland.

MATERIALS AND METHODS

Plot trials for comparison of 42 Italian ryegrass varieties were sown in spring of 1992 at four locations, each with three replications (Lehmann *et al.* 1995). The trials were managed according to standard procedures, with determination of dry mass (DM) yield for each of five cuts during the first full harvest year (1993). First symptoms of *Xanthomonas* were observed during the second growth of 1993 and recorded at two locations (Zürich-Reckenholz and Oensingen) between June 14 and June 16 by using a 1 to 9 visual score scale (1=no disease symptoms, 9=all plants heavily diseased). Yield data of the same two locations were converted to relative values (location mean = 100 %) and pooled with the *Xanthomonas* disease score data to calculate linear regressions either individually for each location, or for the means over both locations.

Observations in a complex experiment with different combinations of red clover (*Trifolium pratense*) and Italian ryegrass cultivars, grown in three replications at Oensingen, were used to assess the influence of the bacterial wilt disease on the competitive ability of Italian ryegrass. Yield proportion of grass and clover was estimated visually for each cut of 1993. According to the disease records of the regular variety trial, two highly resistant (AXIS, diploid and CERVUS, tetraploid) cultivars were identified and compared to two moderately susceptible ones (TURILO, diploid and LIPO, tetraploid). Development of grass proportion over the growing season was compared for the resistant and susceptible cultivars.

RESULTS

Xanthomonas disease scores varied enormously among the varieties tested. The most recent Swiss cultivars (LIPURUS and VICUGNA), selected by using artificial infection techniques, showed the highest level of resistance. There was a striking relationship between the single visual score of *Xanthomonas* and annual DM yield (Fig. 1). Yield declined by 4 % for each unit of the disease score, with a coefficient of linear correlation (r) of -0.88. Nearly the same relationship was observed when the groups of cultivars with disease scores either better or worse than 4.0 were analyzed separately.

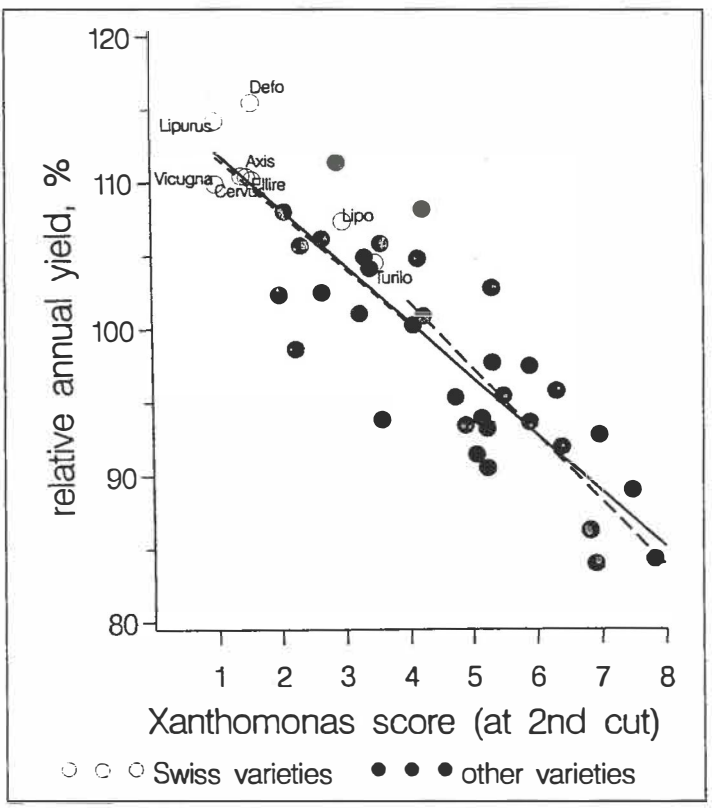


Figure 1. Relationship between *Xanthomonas* disease score (1=no symptoms) given mid-June (during second growth) and annual yield of 42 Italian ryegrass varieties (means of 2 locations). Solid line = linear regression for all varieties (r^2=0.78); dotted lines = linear regression for varieties with disease scores <4 (r^2=0.40) or disease scores >4 (r^2=0.62) respectively.

Analysis of the yield results of single cuts of the first full harvest year showed large differences among the regression coefficients on the *Xanthomonas* score (Table 1).

Yield of the first cut was almost independent of *Xanthomonas* resistance, indicating that yielding ability of all cultivars was intact prior to the incidence of the disease. The impact of the disease then increased rapidly, reaching its highest level during the third growth when yield decreased by 11 % (relative to the experiment mean) for each unit of *Xanthomonas* score. This meant that the most resistant cultivars yielded more than double the amount of dry mass than the most susceptible ones. For the 4th and 5th cuts, the relationship between disease score and yield was much weaker and partly not significant, probably because weed infestation of the plots of the susceptible cultivars led to unrealistic yield results.

Table 1. Coefficients of regression of relative dry mass yield (individual cuts and annual total) on *Xanthomonas* disease scores for two locations

Location	Para-meter	1st cut	2nd cut	3rd cut	4th cut	5th cut	annual total
Reckenholz	b	-0.81	-6.31	-11.29	-1.88	-4.08	-3.67
	r^2	0.09 n.s.	0.65 **	0.73 **	0.06 n.s.	0.15 *	0.64 **
Oensingen	b	-1.08	-5.47	-11.03	-0.32	-1.93	-4.05
	r^2	0.08 n.s.	0.69 **	0.83 **	0.00 n.s.	0.09 n.s.	0.71 **
mean	b	-0.88	-6.00	-11.34	-0.85	-2.61	-3.84
2 locations	r^2	0.11 *	0.75 **	0.83 **	0.03 n.s.	0.15 *	0.78 **

Regression: relative DM yield (%)=a + b *Xanthomonas* score.
r^2: n.s. not significant, * p(r 0) < 0.05, ** p(r 0) <0.01

It was obvious that *Xanthomonas* resistance was also an important factor of stand persistence (Fig. 2). Highly susceptible cultivars (disease scores >5) clearly ranked poorest in persistence as judged at the end of the third year. However, some cultivars of only moderate resistance showed a good capacity of regeneration, enabling them to persist even better than those with the highest level of *Xanthomonas* resistance.

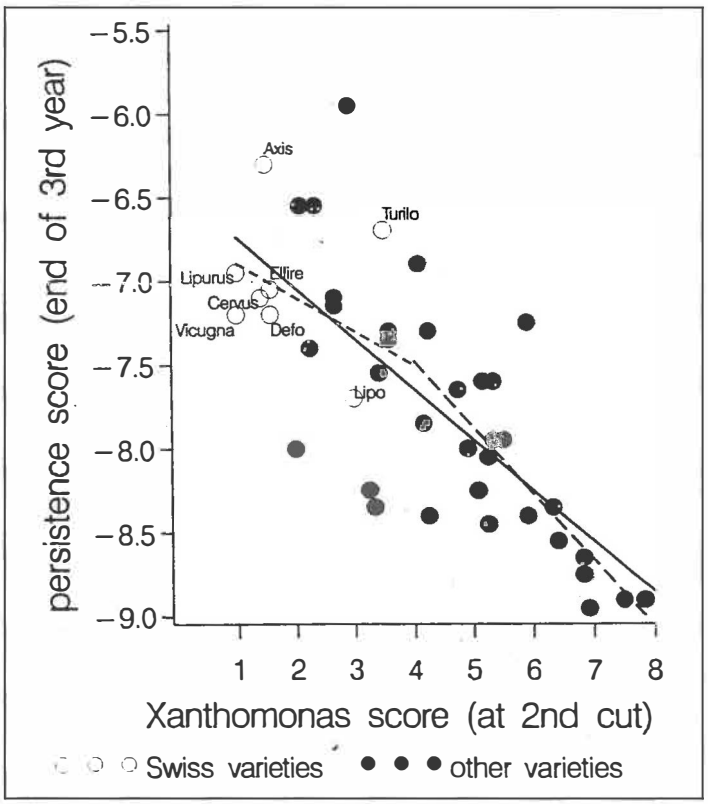


Figure 2. Relationship between *Xanthomonomas* disease score and score of stand persistence (9=poorest persistence, all plants dead) of 42 Italian ryegrass varieties (means of 2 locations). Solid lines=linear regression for all varieties (r^2=0.57); dotted lines=linear regression for varieties with disease scores <4 (r^2=0.08) or disease scores >4 (r^2=0.56)

The comparison of grass monoculture yields during the heavy *Xanthomonas* attack recorded at Oensingen with the behaviour of a choice of varieties grown nearby in a mixture with red clover (Fig. 3) showed that the disease had a marked influence on the competitive ability of Italian ryegrass. As compared to highly resistant varieties, the more susceptible ones lost a greater part of their proportion in the mixture. The effect was more marked and lasted longer than the effect of the disease on grass monoculture yield. A very high level of *Xanthomonas* resistance was required for Italian ryegrass to withstand competition from red clover sufficiently to maintain the desired proportion in the grass-clover mixture.

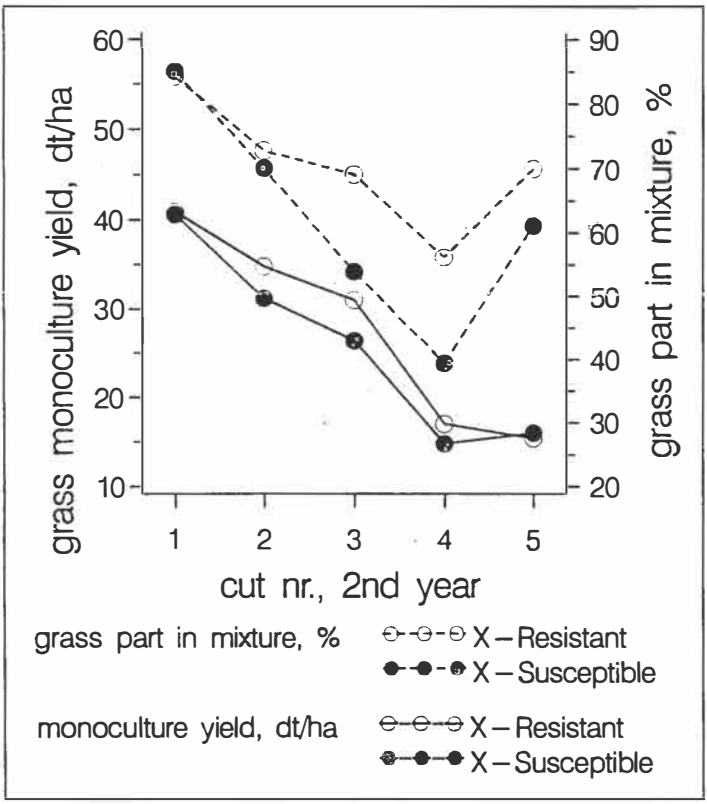


Figure 3. Development of grass part in a mixture with red clover, compared to DM yield of respective grass monoculture, for the mean of two highly *Xanthomonas* (X-) resistant varieties (AXIS and CERVUS) and two moderately susceptible varieties (TURILO and LIPO).

DISCUSSION

Improved disease resistance is often thought to have to be payed for by a decrease in yield, or at least by a delay in developing resistant and at the same time high yielding varieties (Boller, 1991). This was obviously not the case for *Xanthomonas* resistance in this study. Indeed, resistance against *Xanthomonas* was closely and positively correlated with DM yield. Direct effects of *Xanthomonas* on yielding ability are mainly responsible for the favourable yield results of resistant cultivars: The development of affected tillers is stopped when they are heading and are thus in their potentially most productive phase. Even more importantly, regrowth after cutting is severely hindered. This is seen clearly from the in-crease of the relative effects of *Xanthomonas* between the second and the third cut (Table 1).

These results corroborate an earlier, similar study of Schmidt and Nüesch (1980) who were able to classify Italian ryegrass cultivars into a more resistant and a susceptible group.

Our present results reveal a consistent and continuous influence of *Xanthomonas* resistance on yield throughout the full range of disease scores observed. It was also clear that the high level of resistance of the most recent varieties enabled them to outyield the less resistant varieties LIPO and TURILO which had been considered to be among the most resistant varieties in the previous study.

Although a certain level of resistance was necessary for Italian ryegrass varieties to survive the *Xanthomonas* attack and thus to persist beyond the second year, a very high level of resistance was not necessarily correlated with outstanding persistence. This confirms results of Nüesch (1989) who even reported a slight decrease in persistence due to selection for *Xanthomonas* resistance within moderately susceptible cultivars. In a breeding programme aiming at resistant and persistent cultivars, plants selected for *Xanthomonas* resistance must thus be further screened for persistence. This implies that one should avoid too high selection pressures in artificial infection trials in order to allow for further selection among the surviving plants.

Swiss farmers usually grow Italian ryegrass in clover-grass mixtures, very often in a dual mixture with red clover. It is therefore important to know the impact of disease resistance on the behaviour of the Italian ryegrass varieties in a clover-grass mixture. The observations reported here suggest that *Xanthomonas* resistance is an even more important factor of the success of a variety under interspecific competition than when grown in monoculture. This is in contrast to leaf diseases like crown rust whose negative effects, e.g. on forage quality, tend to be reduced in a mixture with a healthy companion species. In order to withstand competition from red clover during typical *Xanthomonas* attacks, the level of resistance of Italian ryegrass cultivars should exceed that of LIPO and TURILO.

It is concluded that resistance against bacterial wilt is a major component of the success of Swiss varieties of Italian ryegrass in our country. While natural infection pressure in the breeding nurseries was the driving force to obtain resistant varieties in the 1970s, the high level of resistance of the more recent cultivars is the result of the systematic use of artificial infection techniques in our breeding programme.

REFERENCES

Boller, B.C., 1991: Yield progress in ryegrass selection programmes focussing on disease resistance, pp. 33-34 in: A.P.M. den Nijs and A. Elgersma (eds.): Fodder Crops Breeding: Achievements, Novel Strategies and Biotechnology. Proceedings of the 16th Meeting of the Fodder Corps Section of EUCARPIA, Wageningen (NL), 18-22 November 1990. Pudoc Wageningen, 230 pp.

Egli, T.; Goto, M.; Schmidt, D., 1975: Bacterial wilt, a new forage grass disease. *Phytopathologische Zeitschrift* **82**, 111-121.

Lehmann, J.; Schubiger, F.X.; Briner, H.-U.; Bassetti, P.; Mosimann, E., 1995: Italienisches Raigras und Bastard-Raigras. *Agrarforschung* **2**, 104-107.

Nüesch, B., 1989: Die Auswirkungen von Resistenzselektionen auf Ertrag und Ausdauer von Rotklee und Italienisch-Raigras. *Landwirtschaft Schweiz* **2**, 195-199.

Schmidt, D., 1976: Différences variétales de sensibilité des graminéees fourragères au flétrissement bactérien causé par *Xanthomonas graminis*. *Revue suisse d'Agriculture* **8**, 99-104.

Schmidt, D.; Nüesch, B., 1980: Resistance to bacterial wilt (*Xanthomonas graminis*) increases yield and persistency of *Lolium multiflorum*. *EPPO Bulletin* **10**, 335-339.

ENDOPHYTES IN MEADOW FESCUE: A LIMITING FACTOR IN RUMINANT PRODUCTION ?

DACCORD R., SCHMIDT DOROTHEA*, ARRIGO Y. and GUTZWILLER A.

SWISS FEDERAL RESEARCH STATION FOR ANIMAL PRODUCTION,
CH-1725 POSIEUX,
*SWISS FEDERAL AGRICULTURAL RESEARCH STATION,
CH-1260 NYON,
SWITZERLAND

ABSTRACT

Two lots of meadow fescue, one being infected with endophyte (*Acremonium uncinatum*) and one being free of endophyte have been tested in a digestibility trial with sheep. Endophyte infestation did not influence the nutrient composition of the test portions. It did however enhance the digestibility of the organic matter and of the cell wall constituents. The ingestion of meadow fescue, with or without endophyte infestation, did neither change the rectal temperature nor the ear temperature of the sheep (indications of vasoconstriction). The concentration levels of the principal metabolites and blood enzymes were not altered.

INTRODUCTION

Endophytes are fungi living inside the plants without causing any disease symptoms. Endophytes of the genus *Acremonium* are frequently found in forage grasses. They might induce resistance to pests and improve vigour and persistence of the plants, such as *Festuca arundinacea* (tall fescue) colonized by *Acremonium coenophialum* as reported from the U.S.A. and *Lolium perenne* (perennial rye-grass) colonized by *A.lolii*, especially in New Zealand.

Presence of endophytes induces alkaloid production and accumulation in the plants; the alkaloids may cause toxicosis to animals consuming the forage. The most dangerous mycotoxins in tall fescue are the ergopeptides, which induce "*fescue summer toxicosis*" and in winter the "*fescue foot diesease*", syndroms characterised by a general sickness, hyperthermia and increased respiration rate, or by gangrenes of the extremities, especially with cattle. Mares develop frequent gestation troubles. The symptoms are due to constriction of the peripheral blood vessels.

Perennial rye-grass often contains a mycotoxin of the lolitrem group, neurotoxins causing "*rye-grass staggers*". The affected animals, especially sheep and cattle, show uncoordinated movements and lack of equilibrium, which may seriously compromise survival.

Meadow fescue (*Festuca pratensis*) has a higher nutritive value and is more extensively used in Switzerland than tall fescue. It is frequently colonized by *A. uncinatum* (Schmidt. 1994). The question was to determine if this endophyte has a negative effect on ruminants.

MATERIAL AND METHODS

Seed lots of endophyte free and infected *F. pratensis* cultivar Predix, produced at the Federal Agricultural Research Station in Nyon, were sown at the Federal Research Station for Animal Production at Posieux. During the second year , forage of the 1rst, 2nd and 4th cut was collected for a digestibility trial with sheep (4 animals per treatment).

RESULTS

The nutrient content of meadow fescue was not significantly influenced by the endophyte (table 1). Slight differences can be explained by a higher percentage of fescue in the endophyte infected plots compared to the endophyte free ones (93 % versus 87 % of green matter, respectively). Endophyte free plots contained more spontaneous white clover, explaining the difference in average crude protein (143 g/kg dry matter in endophyte infected forage compared to 155 g). Analysing pure meadow fescue, Schmidt

and Scehovic (1994) have reported a higher N content in endophyte infected samples, a finding contrary to our results.

Table 1: chemical composition of the meadow fescue without and with endophyte

Cut:		1st	1st	2nd	2nd	4th	4th
Endophyte:		without	with	without	with	without	with
Dry matter	%	15.2	15.0	16.3	16.7	18.3	17.6
In the dry matter:							
Organic matter	g/kg	905	905	896	903	869	876
Crude protein	g/kg	159	148	146	135	159	147
Crude fiber	g/kg	278	290	256	263	237	245
NDF	g/kg	517	519	479	484	459	458
ADF	g/kg	302	314	278	288	259	267
Hemicellulose	g/kg	215	206	201	196	199	191
N-free extract	g/kg	468	468	495	505	473	484
Ash	g/kg	95	95	104	98	131	124
Ca	g/kg	6.2	6.2	5.4	5.8	5.5	7.1
P	g/kg	4.0	4.2	4.1	4.2	4.5	4.9
Mg	g/kg	1.3	1.3	1.8	1.8	1.8	2.0

Alkaloid analyses revealed an average of 1644 g/g of lolines (N-acetyl-plus N-formyl-loline) in endophyte infected samples. These alkaloids are the only that accumulate in *F. pratensis* colonized by *A. uncinatum* (Bush and Schmidt, 1994).

The digestibility of the organic matter, the cell wall constituents and the N-free extract was significantly higher in endophyte infected meadow fescue (table 2). On the other hand the digestibility of crude protein was lower. This reduction could partially be explained by the lower N content of endophyte containing fescue and its smaller proportion of clover, which has a high N-digestibility. The presence of endophyte seems to improve digestibility of the cell wall fraction. Schmidt and Scehovic (1994) have already estimated an increased digestibility of the organic matter in endophyte infected meadow fescue, based on its chemical composition. This positive effect is in contrast with the reduced digestibility of the organic matter observed by Hannah et al. (1990) in tall fescue.

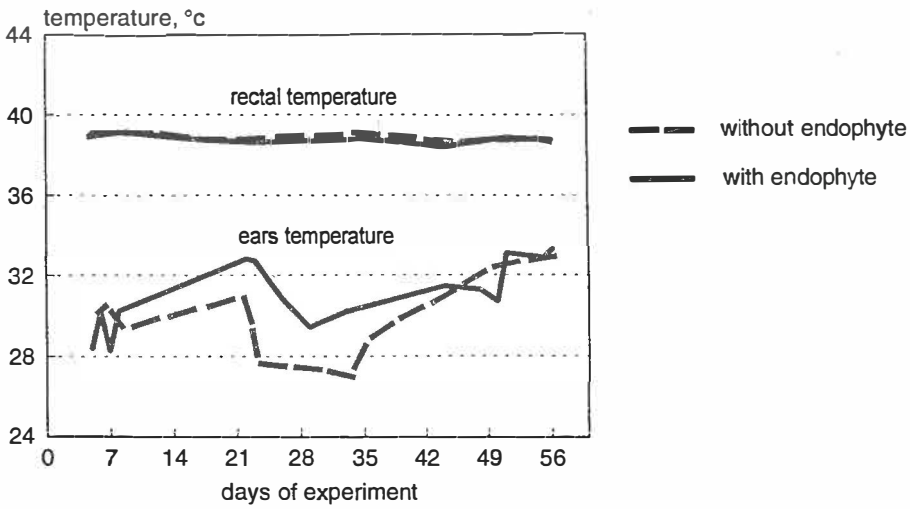
Table 2: digestibility of meadow fescue measured with sheep (means of 1st, 2nd and 4th cuts)

Endophyte:	without	with	SEM
Organic matter	76.2[a]	77.7[b]	0.19
Crude protein	70.4[a]	68.8[b]	0.39
Crude fiber	83.2[a]	85.5[b]	0.41
NDF	81.9[a]	83.6[b]	0.29
ADF	79.6[a]	82.3[b]	0.28
Hemicellulose	84.9	85.5	0.50
N-free extract	74.4[a]	76.1[b]	0.22

Means with different letters in a row are statistically different ($P < 0.05$).
SEM = standard error of means

The ingestion of meadow fescue with or without endophyte by sheep in the digestibility trial did not have any significant effect on their rectal or ear temperature (figure 1). Ear temperature should have been decreased if peripheral vasoconstriction had occurred.

Figure 1: rectal and ears temperature of the sheep involved in the digestibility trial



The intake of endophyte colonized fescue had no effect on the plasma concentration of the principal metabolites and enzymes in the sheep. Plasma prolactin concentration, which is decreased in animals feeding on endophyte colonized tall fescue (Chestnut et al. 1989), was not affected in this trial.

DISCUSSION

The endophyte of the meadow fescue (*Acremonium uncinatum*) did not have significant negative effects on sheep, in contrast to the endophyte of tall fescue (*Acremonium coenophialum*). This result should be confirmed by trials with cattle.

Specific experimental procedures should be developed to measure with accuracy the effects of the different endophytes on the herbivores.

If these effects are not negative, the plant/endophyte symbiotum could be an interesting way to improve the production and the feeding value of grass.

REFERENCES

BUSH L. and SCHMIDT D. 1994. Alkaloid content of meadow fescue and tall fescue with their natural endophytes. IOBC wprs Bulletin, 17, 259 - 265.

CHESTNUT A.B., ERICKSON B.H., McKENZIE P.P., FRIBOURG H.A., McLAREN J.B. and WALLER J.C. 1989. Effect of differing levels of Acremonium coenophialum infection of tall fescue (Festuca arundinacea) on bovine serum prolactin. XVI International Grassland Congress, Nice, 701 - 702.

HANNAH S.M., PATERSON J.A., WILLIAMS J.E., KERLEY M.S. and MINER J.L. 1990. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. J. Anim. Sci., 68, 1693-1701.

SCHMIDT D. 1994. Du nouveau sur les endophytes de la fétuque des prés. Revue suisse Agric., 26, 57 - 63.

SCHMIDT D. and SCEHOVIC J. 1994. Results of one trial examining the influence of Acremonium uncinatum and leaf-spot diseases on different chemical components and nutritive value of festuca pratensis. IOBC wprs Bulletin, 17, 187 - 192.

Acremonium spp. - occurrence in cultivars and collected ecotypes of the genus *Festuca*

EGGESTEIN, ST.; PFANNMÖLLER, MONIKA; SCHÖBERLEIN, W.

Institut für Acker- und Pflanzenbau, Saatgutwirtschaft,
Landwirtschaftliche Fakultät der Martin-Luther-Universität Halle-Wittenberg, Germany

ABSTRACT

Endophytic fungi of *Acremonium*-type have been detected in 22 of 86 seed samples of *Festuca* species so far. The examined material was infected between 1 - 98 % with *Acremonium* endophytes. Seeds from meadow fescue growing in the Beskides revealed an above-average infestation level. Seed was obtained from the Institut für Genetik und Kulturpflanzenforschung Gatersleben, Genbank Malchow, and from the Norddeutsche Pflanzenzucht. The material included seed lots from plants growing in the wild, from botanical gardens and of commercial cultivars. Furthermore, infected seeds were sown and young plantlets screened for the presence of endophytes. Up to now we have succeeded in isolating the fungus from leaf sheaths of young plants of *Festuca rupicola.* After examining the growth rates of the endophytic colonies and morphological traits as size and shape of the conidia, a close resemblance of the isolated fungi to the anamorph of the recently established species *Epichloë festucae* became obvious. There are no reports of choke in the examined material.

Keywords: endophyte, fescue species, evaluation

INTRODUCTION

Acremonium-like endophytic fungi have been isolated from many grass species of the subfamily *Pooideae* which suggests a coevolutionary relationship between these grass hosts and endophytes. Many grasses from the genus *Festuca*, with approximately 450

species one of the largest within the pooid subfamily, are of special interest for forage production on the one hand and turfgrass breeding on the other. Our aim was to evaluate endophyte infestation in fescue species that were collected at different sites in Central and Eastern Europe and in Denmark.

MATERIAL AND METHODS

The 86 seed lots from 23 fescue species examined in this evaluation included seed samples from plants growing in the wild and from botanical gardens as well as 16 commercial cultivars which are stored at the Institut für Genetik und Kulturpflanzenforschung Gatersleben, Genbank Malchow.

Seeds were stained with the rose bengal staining method (Saha et al. 1988). Seed lots that were found to harbour endophytic mycelium were sown and the young plantlets were then screened for the presence of endophytes. For the isolation of the fungus surface-sterilized caryopses or leaf sheaths were placed on potato-dextrose agar (PDA) containing 100 µg/ml penicillin and 100 µg/ml streptomycin and incubated at 22 C in the dark. After 3 weeks hyphae began to grow out of the plant material. Subsequently, the mycelium was microscopically analyzed. The shape and the size of conidia and conidiogenous cells produced *in-vitro* were described.

For the examination of the growth rates 2 mm^2 plugs taken from actively growing colonies were placed on corn-meal agar (CMA), malt-extract agar (MEA) and PDA and inoculated at 16 C, 20 C, 24 C, 28 C, and 32 C. The colony diameter was measured after 2 weeks (16 C - 28 C) and after 4 weeks (32 C).

RESULTS AND DISCUSSION

22 seed samples (25,6%) appeared to harbor *Acremonium*-like endophytic mycelium (Tab. 1 and 2). A high infection rate could be observed in meadow fescue wild material (9 of 14 seed lots) from the Czech republic. Seven seed samples of this material had infection levels >92%, whereas no meadow fescue variety as well as other commercial cultivars were found to contain endophytic mycelium.

Tab. 1: Fescue species screened for the presence of endophytes

Species	Samples (endophyte-infected)	Species	Samples (endophyte-infected)
F. arundinacea	27 (2)	*F. orientalis*	1 (0)
F. brachyphylla	1 (1)	*F. ovina*	2 (0)
F. capillata	1 (1)	*F. paniculata*	1 (1)
F. cinerea	2 (0)	*F. polesica*	1 (0)
F. dolichophylla	1 (0)	*F. pratensis*	14 (9)
F. drymeja	1 (0)	*F. pseudovina*	1 (0)
F. extremorientalis	1 (0)	*F. rubra*	17 (3)
F. gigantea	3 (2)	*F. rupicola*	2 (1)
F. heterophylla	2 (0)	*F. scirpifolia*	1 (0)
F. maghellanica	1 (0)	*F. sibirica*	3 (2)
F. mathewsii	1 (0)	*F. sulcata*	1 (0)
		F. supina	1 (0)

A high endophyte frequence was also evident in other fescue species growing in the wild. Similar results were obtained by Latch et al. (1987) and Cagaš (1991) who found that varieties had a lower infestation rate than ecotypes. These results suggest that material collected in the wild may have a higher rate of endophytic infection compared with seeds under long-term storage as usual in modern agriculture, a process tending to kill the endophyte in the seed. It became also obvious that all seed samples found to be infected by endophytic fungi were obtained either from plants which had been grown from seeds sown soon after collection or from clones originated from these plants. There was in no case extended seed storage prior to sowing.

Tab. 2: Endophyte infection level in fescue species

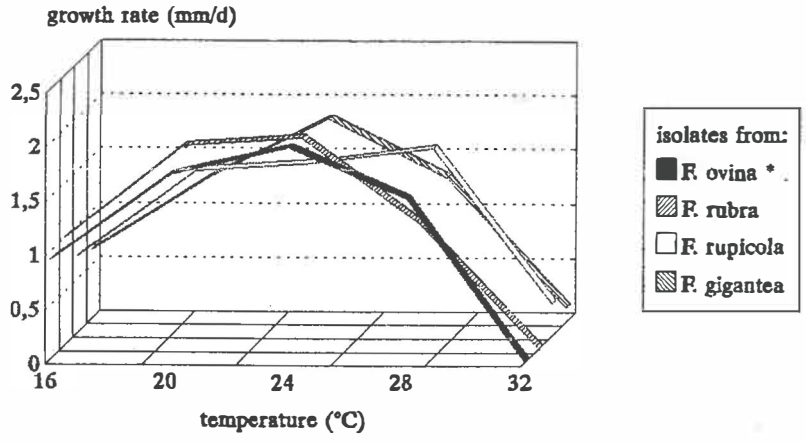
Species	Number of samples	Origin	Infection
F. arundinacea	2	Germany	2% - 3%
F. brachyphylla	1	Denmark	17%
F. capillata	1	Poland	12%
F. gigantea	2	Germany	100%
F. paniculata	1	Germany	19%
F. pratensis	9	Czech Rep.	26% - 100%
F. rubra	1	Germany	60%
F. rubra	2	Czech Rep.	30% - 59%
F. rupicola	1	Russia	100%
F. sibirica	1	Germany	55%
F. sibirica	1	Russia	72%

Apart from the meadow and tall fescue we have so far succeeded to isolate the endophyte of *F. gigantea*, *F. rubra* and *F. rupicola* from leaf sheaths as well as directly from the seed. The growth rates of the endophytic colonies measured at different temperatures are presented in figure 1. The most active fungal growth was observed at 24 C for the isolates from *F. gigantea*, *F. rubra* and the reference strain from *F. ovina*. The endophyte from *F. rupicola*, however, was the only one to have the temperature optimum at 28 C and, together with the endophyte isolated from *F. gigantea*, to show active growth at 32 C.

Small differences in the colony appearance were observed among the isolated endophytes (Tab.3). These colony characteristics are subject to great variation which explains the different growth behaviour observed in this examination. Hence, they are not definite for the identification of fungal endophytes (Christensen 1993).

After examining size and shape of the conidia as well as of the conidiogenous cells, a close resemblance of the isolated fungi to the anamorph of the recently established species *Epichloë festucae* (Leuchtmann et al. 1994) became obvious (Tab. 4).

Figure 1: Colony growth rates of endophytic fungi on PDA in dependence on temperature
Incubation time: 2 weeks (16°C-28°C), 4 weeks (32°C)



Growth rate (literature data) for E. festucae at 24°C on PDA: 21-56 mm/3 weeks (1-2,67 mm/d)
***) The endophyte from F. ovina was used as a reference fungal strain**

Tab. 3: Colony appearance of isolated fungal strains from different fescue species

Medium	Fescue species		
	F. gigantea	*F. rubra*	*F. rupicola*
PDA	raised, slightly wrinkled, cottony; white; reverse yellowish-brown	slightly raised, wrinkled, felty; yellowish-white; reverse tan	slightly raised, wrinkled, cottony; white; reverse brownish-tan
MEA	slightly raised, cottony; white; reverse yellowish	raised, cottony; white; reverse tan	slightly raised, cottony; white; reverse creme-coloured
CMA	flat, compact, smooth; milky; reverse milky	flat, compact, smooth; milky; reverse milky	flat, compact, smooth; milky; reverse milky

Tab.4: Morphology of conidia and conidiogenous cells of fungal endophytes in comparison with literature data

literature data / isolate	conidiogenous cells (length)	conidium size
literature *Epichloë festucae* (Leuchtmann et. al. 1994)	12-25 µ	4,7 µ x 2,2 µ
isolate from *F. gigantea*	12-22 µ	5 µ x 3 µ
isolate from *F. ovina* *)	11-13 µ	5 µ x 3 µ
isolate from *F. rubra*	10-17 µ	3-4 µ x 2-3 µ
isolate from *F. rupicola*	10-16 µ	3-5 µ x 3 µ

*) The endophyte from *F. ovina* was used as a reference fungal strain.

SUMMARY

Caryopses of 86 seed samples from different fescue species have been examined, 22 of these contained endophytes. A high infection rate was observed in Czech material growing in the wild with 7 seed lots having an endophyte infection >92%. No commercial cultivar obtained from the genebank was endophyte-infected. Long-term seed storage in modern agriculture may be responsible for this result. Morphology of conidia and conidiogenous cells as well as colony appearance fit with the description of *Epichloë festucae*, the endophyte of fine fescue grasses. There are, however, differences in growth rate characteristics. Some of the fescue species examined in this evaluation are used for turf grass mixtures under dry conditions. Endophyte infection may contribute to better performance concerning vigour, drought and pest resistance.

LITERATURE

Cagaš, B. 1991: Genetic resources of tall fescue (*Festuca arundinacea* Schreb.) und *Acremonium coenophialum*. Report of a working group on forages. Budapest, 28.-30. Oktober 1991, 76-81

Christensen, M.J. 1993. Practical guide to the identification of *Acremonium* endophytes. Proc. Second Int. Symp. *Acremonium*/Grass Interactions. Eds. Hume, Latch & Easton, 37-38

Latch, G.C.M., Potter, L.R. and B.F. Tyler 1987. Incidence of endophytes in seeds from collections of *Lolium* and *Festuca*. Ann. appl. Biol., 111, 59-64

Leuchtmann, A.; Schardl, C.L. and M.R. Siegel 1994. Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. Mycologia, 86 (6), 802-812

Saha, D.C., Jackson, M.A. and J.M. Johnson-Cicalese 1988. A rapid staining method for detection of endophytic fungi in turf and forage grasses. Phytopathology, 78, 237-239

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Mrs. Gaue (Norddeutsche Pflanzenzucht) and Mrs. Willner (Institut für Genetik und Kulturpflanzenforschung, Genbank Malchow) for providing the material.

Contrasting palatability in endophyte free and infected tall fescue did not result in changes of intake and animal growth

J.C. EMILE, M. GHESQUIERE and J. GONDRAN

STATION D'AMELIORATION DES PLANTES FOURRAGERES, INRA 86600 LUSIGNAN - FRANCE.

Farmers and seed firms in Europa are more and more questioned on the potential effect, either harmful or beneficial, of the endophytic fungus *Acremonium sp,* naturally occuring in grasses. The effects of *A. coenophialum* in tall fescue on both plant growth and beef cattle performances in USA are now well documented (see the recent reviews of Joost, 1995 and Paterson et al, 1995). However, in European countries, only a few number of reviews or observations have been conducted (Raynal, 1991; Lewis 1995 in this conference). Therefore, the objective of this paper is to provide first animal references with livestock fed with infested forage under representative conditions of the level of intensification in Western Europa.

Material and methods

Two fields (2 ha each) were established in the INRA Plant Breeding Station at Lusignan (France, 0.15° E, 45.26° N) in Autumn 94 with the same mid-late variety of tall fescue (Cv Clarine) but differing in the rate of endophyte infestation: 0 % = E-; 100 % = E+. Successives trials have been planned to investigate the possible effect of infestation on cattle. For the first one, a late hay cutting was done in June 1995 in order to maximise the potential toxic effect of the endophyte in the forage. E+ and E- hays were then assessed by animals and the first results are given hereafter.

Palatibility of the two forages was compared with 'Texel' sheep using «cafeteria tests» (Gillet and *al.,* 1983). Sheep were allowed to choose between 15 pairs of troughs filled with 500 g DM of either genotype. The rate of consumption of each forage after 3 hours is considered as a good indicator of palatability. Four replications (A, B, C and D) were done with forage coming from different roundballs. Furthermore, dairy 'Holstein' heifers

were fed *ad libitum* with E- or E+ hay in a crossed trial of 5 weeks and 12 animals per batch. As heifers were intended to be used as a high performing dairy cattle on the following year, they were given 1.7 kg DM a day of concentrates. Forage intake (4 days a week) as well as average daily gain (ADG) were recorded.

Results

Forage palatability

Along the 4 replications, the preference of sheep was clearly on behalf of the endophyte-free hay (figure 1). The mean rate of consumption was 53.1 vs 15.7 % for E- and E+ resp.

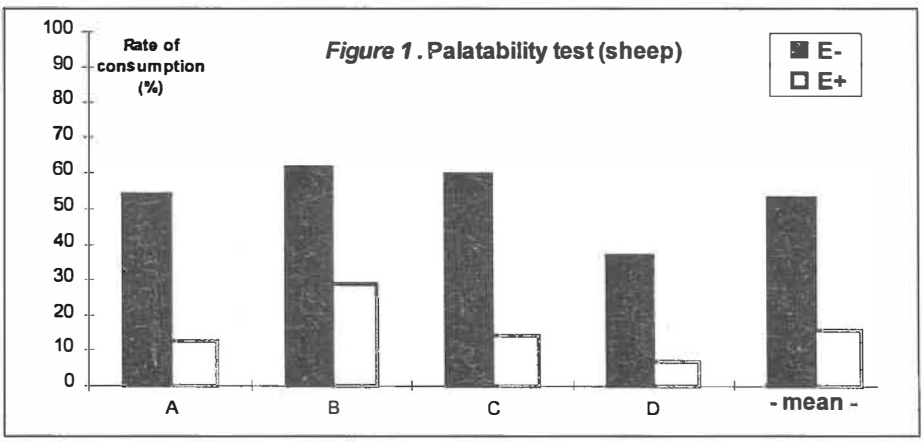
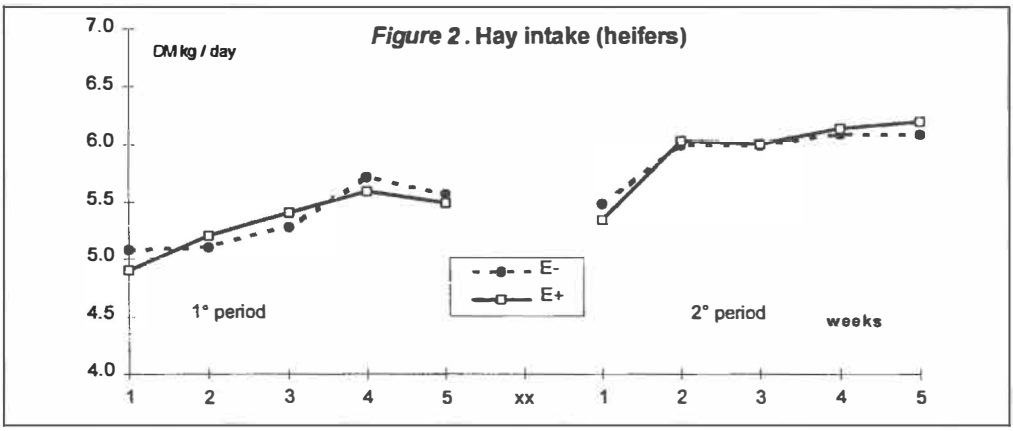


Figure 1. Palatability test (sheep)

Heifers performances

Both E- and E+ hays were similar regarding chemical composition (88.9 % dry matter, 92.1 % organic matter, 10.9 % crude protein and 29.4 % crude fiber). During the 10 weeks of the trial, E- and E+ hay intake were very close as shown in figure 2. The mean value of daily intake was respectively 5.65 and 5.63 kg DM for E- and E+. The average daily gain in the second part of the trial (707 g) was lower than in the first part (1006 g) but no significant differences occurred between E- and E+ hays wich allowed respectively a 874 and 838 g/d ADG.



Discussion

Thus, presence of the endophytic fungus in tall fescue hay induced quite different effects on palatability and intake. The endophyte is known to release alkaloids at a high concentration rate in grasses, especially when cut during heading time; this could explain the broad difference observed in palatability between E- and E+ hays. Attention in previous studies has been mostly focused on the effect of specific ergopeptidic compounds such as ergovalin on health and productivity of animals. According to Fribourg estimations (Fribourg et *al.,* 1991), i.e. a decrease of 45 g/d ADG by increasing of 10% the rate of infestation, and considering that hay represented 2/3 of the total energy value of the total diet, 1000 g/d ADG for E- vs 700 g/d ADG for E+ should have been expected in this trial. However, our results indicated that, when no choice was allowed, the endophyte did not affected growth of high yielding heifers nor intake. Apparently, the alkaloids involved in palatability would have only very little effects, if any, on intake, growth or health of the animals, or would require to be at much more high levels in forage. Environmental conditions and feeding management (concentrate given to heifers) could also have decreased the potential negative effect of the endophyte on animals and could explain that the results were in discordance with North American studies. Also, twice 5 weeks of feeding with infested hay might have been too short to point out obvious toxic effects. Dosage of ergovaline in samples of both hays and prolactine in blood of the heifers, are in progress to detect possibly more subtle effects on the health of animals. As

first conclusions, i) breeding for feeding value in tall fescue, which is currently based on palatability tests, should now include joint assessment of the rate of infestation by the endophyte and ii) feeding dairy cattle with tall fescue even highly infested with endophyte was no problem under our experimental conditions as it would have been likely under any similar intensive management.

References

Fribourg H.A., Hoveland C.S., Codron P. 1991 La fétuque élevée et *l'Acremonium coenophialum.* Aperçu de la situation aux Etats-Unis. Fourrages, 126, 209-223.

Gillet M., Noël C., and Jadas-Hécart J. 1983. La cafetaria d'auges, méthode d'étude de l'appétibilité des fourrages. Agronomie 3 (8), 817-822.

Joost R.E. 1995. *Acremonium* in fescue and ryegrass: boon or bane? A review. J. Anim. Sci., 73, 881-888

Lewis G. 1995. A review of research on endophyte fungi worldwide, and its relevance to European grassland, pastures and turf. 2nd conference on harmful and beneficial microorganisms in grassland, pastures and turf, Paderborn, November 22-24

Paterson J., Forcherio C., Larson B., Samford M., and Kerley M.1995. The effects of fescue toxicosis on beer cattle productivity. J. Anim. Sci., 73, 889-898

Raynal G. 1991. Observations françaises sur les *Acremonium,* champignons endophytes des graminées fourragères. Fourrages, 126, 225-237

Gene- and Biotechnology

Genetic and biological diversity of grass endophytes

Adrian Leuchtmann

Geobotanisches Institut ETH, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

Abstract

Among the divers assemblage of endophytes that are found in a large number of different plants, a taxonomically defined group of fungi referred to as grass endophytes is the best known. These fungi have been implicated in toxicosis of cattle, but at the same time are believed to have a mutualistic relationship with their hosts under certain conditions. Benefits for the host plant include increased growth, insect resistance and drought tolerance. Grass endophytes of the genus *Epichloë* and the related asexual *Acremonium* forms are widespread in European grasses.

Isozyme markers were used to assess genetic diversity of *Acremonium* endophytes. These analyses revealed that considerable genetic variation was present in most endophytes from wild grass populations, but that far less variation can be expected from cultivated grass species. Moreover, many of the asymptomatic endophytes are assumed to be derived from interspecific hybrids which represent an additional source of genetic variation.

Mating studies among *Epichloë* from different hosts and between *Epichloë* and asexual endophyte strains indicated the presence of several distinct mating populations. Experimental mating populations represent biological species and may include both sexual and asexual strains from different hosts.

The natural diversity of endophytes and its gene pool from wild grasses should be explored and utilized for improvement of infected pasture or turf grasses. However, since experimental transfer of endophytes into new hosts is constrained by limited compatibility and persistence of endophytes in novel associations, modification or transfer of specific genes might be more promising.

Key words: endophytes, isozymes, mating compatibility, *Acremonium, Epichloë*

Introduction

The term endophyte in its broadest sense has been applied to all organisms that are living inside of plants or colonize their tissues without causing symptoms or harm to their hosts. Among the divers assemblage of fungal endophytes that are known from many different plants, the so called 'grass endophytes' which are ascomycetes in the family Clavicipitaceae are probably the best known of the endophytes. This has several reasons: they have been implicated in toxicosis of cattle and sheep (Stuedemann and Hoveland 1988; Prestidge 1993), and they may increase biotic and abiotic stress tolerance of their host grasses, mainly under adverse conditions (Bacon et al. 1977; Clay 1988; Arachevaleta et al. 1989), or enhance vegetative growth (Latch et al. 1985; Clay 1987). The latter aspects, although not very well documented for grasses in Europe, indicate that they could be beneficial to grasses of pastures and turf. However, we are still lacking a thorough understanding of the biology of the endophytes and their interactions with the host plants that would allow us to take full advantage of the biotechnological potential inherent in these interesting associations.

Any successful endophyte biotechnology depends on the recognition of the genetic and biological diversity of the organisms involved, and this will be the topic this paper.

Biology and Life Cycle

Grass endophytes include six genera with approximately 60 species currently recognized world wide (White 1994a), but only the choke-inducing genus *Epichloë* is known from European grasses together with its related asexual forms placed in section *Albo-lanosa* of the form genus *Acremonium*. Although these fungi are referred to two different genera, they simply denote alternative life histories of closely related organisms (Schardl et al. 1991; An et al. 1992).

During the vegetative phase of grass development, *Epichloë* (and *Acremonium*) endophytes grow intercellularly in above-ground plant parts and systemically invade all vegetatively formed tillers. At the time of host flowering, the endophyte may exhibit two alternative modes of reproduction: (1) sexual reproduction of the endophyte by stroma formation whereby host plants are sterilized (choke disease), (2) asexual reproduction of the endophyte by infecting host ovules and becoming incorporated into seeds. The mode of reproduction varies among different grass—fungus associations, or different fungal or grass genotypes. Reproduction may be sexual only in many *Epichloë* species (e.g. *E.*

typhina, *E. clarkii* and *E. baconii*), asexual only in a number of *Acremonium* species from pasture grasses (*A. coenophialum, A. lolii, A. uncinatum*), or intermixed with both types of reproduction on the same or different plants (e.g. *E. festucae*). The mode of fungal reproduction is affecting the ecological fitness of its host grass due to reduced or absent seed production.

Genetic diversity

Genetic diversity of grass endophytes has been assessed by electrophoresis of isozymes in a number of studies. Isozymes (or allozymes at the same locus) are functionally identical enzymes which vary in their biochemical composition within species and can be detected based on different migrations on a gel. Usually 10 to 12 enzymes were selected with specific structural functions providing a conservative estimate of genetic variability, unlike inducible enzymes or noncoding DNA sequences.

Table 1. Isozyme diversity in *Acremonium* endophytes isolated from different host grasses.

Host species	Polymorphic loci (%)	No. genotypes	No. isolates	Ratio	Reference[a]
Agropyron caninum	66.7	2	6	0.333	(1)
Bromus benekenii	41.7	4	22	0.182	(1)
Bromus ramosus	-	1	15	0.067	(1)
Elymus europaeus	58.3	2	41	0.049	(1)
Festuca altissima	-	1	13	0.077	(2)
Festuca arundinacea	90.9	9	69	0.130	(3, 5)
Festuca gigantea	16.7	3	21	0.143	(2)
Festuca heterophylla	16.7	2	2	1.000	(2)
Festuca longifolia	16.7	3	8	0.375	(2)
Festuca obtusa	90.0	5	14	0.357	(3)
Festuca ovina	41.7	2	9	0.222	(2)
Festuca pratensis	9.1	2	17	0.118	(2, 5)
Festuca pulchella	8.3	2	20	0.100	(2)
Lolium perenne	63.6	8	28	0.286	(3, 5)
Melica ciliata	41.7	5	48	0.104	(4)
Sphenopholis nitida	70.0	2	2	1.000	(3)
Sphenopholis pallens	10.1	2	3	0.667	(3)

[a] (1) Leuchtmann 1992; (2) Leuchtmann 1994a; (3) Leuchtmann and Clay 1990; (4) Leuchtmann, unpublished data; (5) Christensen, Leuchtmann et al. 1993

Table 1 summarizes the allozyme variation found in the *Acremonium* endophytes of 17 grass species isolated mainly from wild grasses collected from their natural habitat. Polymorphism was found in isolates of all hosts with two exceptions, *Bromus ramosus* and *Festuca altissima* which appeared to be clonal. Because of the unequal sample sizes among hosts, the ratio of the number of genotypes per number of isolates can be taken as a relative measure for variation. This value ranged from 1.0 to almost 0 with an average of 0.3 indicating that every third isolate of the total sample was genetically different.
In contrast, diversity of endophytes isolated from plants of intensively managed grassland or from cultivars was generally much lower. For example, 90 % of the 52 isolates of *A. coenophialum* from tall fescue collected in the United States were of the same isozyme genotype (Leuchtmann and Clay 1990). Likewise, all 12 isolates of *A. uncinatum* from meadow fescue collected from pastures in Switzerland or from cultivars were identical (Leuchtmann 1994a). It seems that unwitting selection of certain infected plant material by breeders and intensified agricultural practices lead to impoverishment of the natural diversity of endophytes. Many endophytes of the common pasture grasses of temperate zones, including the two species mentioned above, show an increased diversity
when collected at more natural sites or at the border of their main distribution. Among collections of tall fescue and perennial ryegrass originating from locations in southern Europe, North Africa, New Zealand and others, nine or eight different endophyte genotypes, respectively, were detected (Christensen et al. 1993). Many of these endophytes were so distinct that they were tentatively assigned to different taxonomic groupings (TG) within the previously described prevalent species of the respective host. Moreover, genotypes or TG's were often correlated with a specific pattern of alkaloids produced in infected plants. These endophytes might eventually be treated as separate species, when more information on their distribution and biology becomes available.

Interspecific hybridization

The complex multibanded allozyme patterns found in many of the asymptomatic *Acremonium* endophytes suggest that they have duplicated genes which most likely are the result of past hybridization events between sexual or asexual endophytes (Leuchtmann 1994b). Such an example is an endophyte isolated from perennial ryegrass named LpTG-2. It is postulated to be a heteroploid derived from a interspecific hybrid between *A. lolii* and *E. typhina* from perennial ryegrass which has been formed by

parasexual processes in a plant co-infected by the two endophytes (Schardl et al. 1994). The LpTG-2 isolates had two copies of seven enzymes, and the profiles for five of them were exact composites of those from the two suggested parents (Table 2). Further evidence for this hypothesis is provided by gene sequences of the two ß-tubulin genes, the mitochondrial DNA and the rRNA of LpTG-2 which were related to either *A. lolii* or *E. typhina*, or were composites of the two species (Schardl et al. 1994). Such interspecific hybridization may be a common and important mechanism for genetic variation in *Epichloë* and *Acremonium* endophytes.

Table 2. Allozyme profiles of *Epichloë* and *Acremonium* isolates from perennial ryegrass (data from Schardl, Leuchtmann et al. 1994).

Taxon	ACO	ALD	LAP	MDH-1	PGI	PGM-1	PGM-2
LpTG-2 (hybrid)	84/100	79/100	84/97	100/275	78/106	92/100	87/100
A. lolii (LpTG-1)	84	79	84	100	100	97	100
E. typhina (E8)	100	100	97	275	106	100	87

Numbers indicate allozyme migration relative to that of a reference strain. Enzymes are aconitase (ACO), aldolase (ALD), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM). Only homomeric bands are indicated.

Host specificity

An important aspect of the biology of endophytes is their host relation and specificity. Earlier studies have shown that experimental transfer of *Acremonium* endophytes to new host plants was possible generally only within the same or between closely related host species (Leuchtmann 1994b). However, in most artificial infections, persistence of new associations was only monitored for a short time, and endophyte infection was sometimes found to be lost after a prolonged time.

Christensen (1995) has looked at variations in the compatibility of seven taxonomic groupings (TG's) of *Acremonium* from perennial ryegrass, tall fescue and meadow fescue with these three grasses. Examinations of inoculated seedlings were performed after six weeks and again after several month growth in the greenhouse, but no seeds were checked for seed-transmission of the endophytes. In most of the combinations, at least short-term, systemic associations were formed indicating that the endophytes of the three host grasses were not specific to their natural host. However, in many of the novel combinations one or several types of incompatibility reactions were observed, which

included (1) death of highly vacuolated hyphae in young leaf sheath tissues, (2) necrosis at the base of stunted tillers which later died, and (3) loss of infection in some tillers. These findings together with data from previous studies provide evidence that compatible associations between *Acremonium* endophytes and their host grasses are probably determined on the level of individual genotypes, and that long-term persistence of nonhost combinations is rarely achieved (Table 3). The only endophyte which has been demonstrated to be seed-transmitted in nonhost grass species is *E. festucae* (formerly *A. typhinum*) artificially inoculated into *Festuca arundinacea* and *Lolium perenne* (Latch and Christensen 1985). Stable associations of *Acremonium* endophytes with a host grass should meet two criteria: (1) permanent infection of all tillers of a plant, (2) seed-transmission over at least two generations.

Table 3. Compatibility and persistence of *Acremonium* and *Epichloë* endophytes with nonhost grasses of the genera *Festuca* and *Lolium*.

Fungus	Natural host	Inoculated host	Persistence
A. coenophialum	*F. arundinacea*	*L. perenne*	limited, hyphal death
		F. pratense	not known
A. lolii	*L. perenne*	*F. arundinacea*	not known
		F. pratense	limited, loss of infection
		L. multiflorum	not known
		L. temulentum	not known
A. uncinatum	*F. pratensis*	*F. arundinacea*	limited, necrosis
		L. perenne	limited, loss of infection
E. festucae	*F. rubra*	*F. arundinacea*	seed-transmitted
		F. pratense	not known
		L. perenne	seed-transmitted

Data compiled from Christensen 1995; Latch and Christensen 1985; Latch et al. 1988; Siegel et al. 1990

Sexual compatibility

Epichloë has a strict heterothallic mating system requiring the transfer of conidia (spermatia) from one stroma to another of opposite mating type for fertilization (White and Bultman 1987). Mating compatibility has therefore become an important concept for the definition of biological species in the genus *Epichloë*. Until recently, only one species, *E. typhina*, has been recognized on grasses of temperate zones. However, mating tests between *Epichloë* strains from different host grasses showed that not all strains were

compatible among each other, but form different mating populations that represent different biological species. Based on these findings and on morphological studies, several new species have now been described which differ in their host range (White 1993; 1994b; Leuchtmann et al. 1994). *Epichloë typhina* appears to be a generalist species infecting a number of different grass genera, whereas other species are restricted to only one host genus.

It is possible also to perform mating tests between *Epichloë* species and asexual *Acremonium* endophytes by applying conidia produced in culture with a sterile needle onto the surface of a stroma. Using this technique, asexual *Acremonium* endophytes from four different *Festuca* hosts were used to fertilize *E. festucae* from *F. rubra*. All pairings among isolates from *Festuca* using opposite mating types resulted in sexual reproduction and viable ascospores, whereas pairings between mating populations from other host grasses failed (Table 4). Thus, asexual and sexual forms of the same biological species (*E. festucae*) may occur on different grass hosts but are clearly linked by mating compatibility. These results may have important implications for these and other asexual *Acremonium* endophytes, if sexual recombination would be a strategy for strain improvement.

Table 4. Mating tests between stroma forming (S) and non-stroma forming (NS) isolates of *Epichloë festucae*, and between other mating populations represented by *Epichloë* spp. from *Dactylis, Bromus, Brachypodium* and *Agrostis.*

	Host of stromatal parent						
Host of spermatial parent	*F. rubra*	*D. glomerata*	*B. erectus*	*B. sylvaticum*	*A. tenuis*	Symptoms	Habitat
Festuca rubra	A	N	N	N	N	S/NS	Grassland
Festuca ovina	A	-	-	-	-	NS	Grassland
Festuca longifolia	A	-	N	N	-	NS	Grassland
Festuca pulchella	A	-	-	-	-	NS	Alps
Festuca gigantea	A	-	-	-	-	NS	Woods

A = sexually compatible with viable ascospores produced
N = no reaction
- = missing data

Outlook on potential biotechnological modifications of grass endophytes

Endophyte-grass associations represent a unique biological system because of the intimate and systemic relation of the two partners, and the possibility of the endophyte to shift from a completely asymtomatic life style to one with overt disease expression in different associations. An important aspect to bear in mind when dealing with the system is its dual nature. The fungal genotype can influence plant characteristics in various ways, some effects may be desirable while others are detrimental for agricultural practices. At the same time the plant genotype may modify the expression of certain fungal traits such as alkaloid production. It is therefore important to consider both partners when trying to modify a endophyte—grass association. Possibilities for biotechnological modifications include:

1) Novel host/fungus combinations using the vast resources of germplasm present in wild grasses and its endophytes. This may, however, be possible only within the same or closely related host species with no guarantee for long-term persistence of new associations.

2) Use of multiple endophyte genotypes in grass cultivars. This would increase the genetic diversity and possibly enhance the overall fitness of a population.

3) Augmentation of beneficial characteristics such as drought or herbivore resistance by gene transformation or sexual recombination.

4) Elimination of undesired characteristics such as production of alkaloids toxic to livestock using techniques of gene disruption.

It should be emphasized, however, that any manipulation of the endophyte—grass symbiosis may harbor unexpected risks for the environment or may not receive public acceptance.

References

An, Z.-Q., Liu, J.-S., Siegel, M. R., Bunge, G., and Schardl, C. L. 1992. Diversity and origins of endophytic fungal symbionts of the North American grass *Festuca arizonica*. Theoretical and Applied Genetics 85: 366-371.

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

Bacon, C. W., Porter, J. K., Robbins, J. D., and Luttrell, E. S. 1977. *Epichloë typhina* from toxic tall fescue grasses. Applied and Environmental Microbiology 34: 576-581.

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.

Christensen, M. J. 1995. Variation in the ability of *Acremonium* endophytes of perennial rye-grass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and meadow fescue (*F. pratensis*) to form compatible associations in the three grasses. Mycological Research 99: 466-470.

Christensen, M. J., Leuchtmann, A., Rowan, D. D., and Tapper, B. A. 1993. Taxonomy of *Acremonium* endophytes of tall fescue (*Festuca arundinacea*), meadow fescue (*Festuca pratensis*), and perennial rye-grass (*Lolium perenne*). Mycological Research 97: 1083-1092.

Latch, G. C. M., and Christensen, M. J. 1985. Artificial infection of grasses with endophytes. Annals of Applied Biology 107: 17-24.

Latch, G. C., Hunt, W. F., and Musgrave, D. R. 1985. Endophytic fungi affect growth of perennial ryegrass. New Zealand Journal of Agricultural Research 28: 165-168.

Latch, G. C. M., Christensen, M. J., Hickson, R. E. 1988. Endophytes of annual and hybrid ryegrasses. New Zealand Journal of Agricultural Research 31: 57-63.

Leuchtmann, A. 1992. Systematics, distribution, and host specificity of grass endophytes. Natural Toxins 1: 150-162.

Leuchtmann, A. 1994a. Isozyme relationships of *Acremonium* endophytes from twelve *Festuca* species. Mycological Research 98: 25-33.

Leuchtmann, A. 1994b. Isozyme characterization, persistence and compatibility of fungal and grass mutualists. In: C. W. Bacon and J. F. White, Jr. (eds.), Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton, FL, U.S.A., pp. 21-33.

Leuchtmann, A., and Clay, K. 1990. Isozyme variation in the *Acremonium/Epichloë* complex. Phytopathology 80: 1133-1139.

Leuchtmann, A., Schardl, C. L., and Siegel, M. R. 1994. Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. Mycologia 86: 802-812.

Prestidge, R. A. 1993. Causes and control of perennial ryegrass staggers in New Zealand. Agriculture, Ecosystems and Environment 44: 283-300.

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

Schardl, C. L., Leuchtmann, A., Tsai, H.-F.,Collett, M. A., Watt, D. M., and Scott, D. B. 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen *Epichloë typhina*. Genetics 136: 1307-1317.

Siegel, M. R., Latch, G. C. M., Bush, L. P., Fannin, F. F., Rowan, D. D., Tapper, B. A., Bacon, C. W., and Johnson, M. C. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. Journal of Chemical Ecology 16: 3301-3315.

Stuedemann, J. A., and Hoveland, C. S. 1988. Fescue endophyte: history and impact on animal agriculture. Journal of Production Agriculture 1: 39-44.

White, J. R., Jr. 1993. Endophyte-host associations in grasses. XIX. A systematic study of some sympatric species of *Epichloë* in England. Mycologia 83: 444-455.

White, J. R., Jr. 1994a. Taxonomic relationship among the members of the Balansieae (Clavicipitaceae). In: C. W. Bacon and J. F. White, Jr.(eds.), Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton, FL, U.S.A., pp. 3-20.

White, J. R., Jr. 1994b. Endophyte-host associations in grasses. XX. Structural and reproductive studies of *Epichloë amarillans* sp. nov. and comparisons to *E. typhina*. Mycologia 86: 571-580.

White, J. F., Jr., and Bultman, T. L. 1987. Endophyte-host associations in forage grasses. VIII. Heterothallism in *Epichloë typhina*. American Journal of Botany 74: 1716-1721.

MORPHOLOGICAL AND MOLECULAR VARIABILITY AMONG ACREMONIUM ISOLATES FROM 22 SPECIES OF POACEAE IN FRANCE

NAFFAA Walid, GRAND-RAVEL Catherine, GUILLAUMIN Jean-Jacques

Institut National de la Recherche Agronomique (INRA),
Centre de Clermont-Ferrand, 12 Avenue du Brézet, 63039 Clermont-Ferrand, FRANCE

INTRODUCTION

Fungi belonging to genus *Acremonium* are common endophytes of the vegetative and reproductive parts of many species of the family Poaceae. These fungi are responsable for toxicoses on grass-eating mammals (BACON et al. 1977). On the other hand, they increase the resistance of their hosts to various stresses of biotic and abiotic origin (CLAY 1990, LEWIS and DAY 1993, WEST et al. 1993, BREEN 1994). The different effects, both benefic and pernicious, of these endophytes, are the cause of the recent interest taken in these fungi by mycologists and plant breeders.

The first observations of the fungus in the seeds of darnel (*Lolium temulentum*) were effected in 1898 both by VOGL in Germany and by GUERIN in France; the first observation of the endophyte in the vegetative parts of the Graminaceae was carried out by SAMPSON (1933), who established the close proximity between the endophytes of grasses and the Ascomycetous parasite *Epichloë typhina*, the agent of the "choke disease" of Graminaceae.

However, it is only in the recent period that some attention was paid to the taxonomy of both the endophytic *Acremonium* and the related *Epichloë sp*.

The endophytes of the three species of economical interest *Lolium perenne*, *Festuca pratensis* and *F.arundinacea* were the subject of a number of studies in the eighties. These studies were based first upon the size and shape of the spores and conidiophores, then upon physiological characteristics, isozyme patterns and molecular markers. A section "*Albolanosa*" of genus *Acremonium* harboured three species: *Acremonium coenophialum*, the endophyte of *F.arundinacea* (MORGAN-JONES and

GAMS 1982), *A.lolii* from *L.perenne* (LATCH et al. 1984) and *A.uncinatum* from *F.pratensis* (GAMS, PETRINI and SCHMIDT 1990). However, a considerable variability was found within the first two species (CHRISTENSEN and LATCH 1991, CHRISTENSEN et al. 1991). Studies using isoenzymatic markers and alkaloid production pattern (LEUCHTMANN and CLAY 1990, CHRISTENSEN et al. 1993, LEUCHTMANN 1994), distinguished three different taxonomic units within "*A.coenophialum*" and 2 within "*A.lolii*".

Most of studies dealing with endophytes of wild species of Poaceae were carried out in North America by WHITE and his collaborators. Four additional species of *Acremonium* were established from these studies (WHITE and MORGAN-JONES 1987a, 1987b, WHITE et al. 1987, MORGAN-JONES and WHITE 1990).

The studies on endophytes of European Poaceae were less numerous. LATCH et al. (1988) isolated *Acremonium* endophytes from 6 annual species of *Lolium*, but did not describe the isolates from the different species. LEUCHTMANN (1994) compared *Acremonium* isolates from twelve European *Festuca* species for 11 isoenzymatic markers.

As regards *Epichloë*, although the high variability of *E.typhina* had long been recognized, it is only in the recent period that the species was subdivided into several biological species: *Epichloë clarkii, baconii*, and *E.festucae*, respectively parasites of *Holcus sp.*, *Agrostis sp.* and the small fescues, were described by WHITE (1993) and LEUTCHMAN et al.(1994) and separated from *E.typhina sensu stricto*. An important biological distinction was made by WHITE (1988) who showed that in addition to the parasitic species developing a stroma on the host (type I) and the strictly endophytic species, always lacking a stroma ("type III"), an intermediate behaviour exists ("type II") when the sexual stage of the fungus is developed only on a minority of tillers.

So a taxonomy of the *Acremonium* / *Epichloë* group is presently emerging. However, if a framework now exists, it is likely that much remains to be discovered. Especially, as Europe is the centre of origin of all the important forage species cultivated throughout the world, a large diversity can be expected among the endophytes of European grasses.

The "Unité de Mycologie" of INRA at Clermont-Ferrand has recently undertaken a study of the *Acremonium* isolates of cultivated and wild French species of *Lolium* and *Festuca*. Our purpose is to describe the variability of these endophytes and, if possible, to

have some contribution in the progressing taxonomy of the group. The long-term objective is to provide the grass breeders with a knowledge of the isolates allowing the selection of the most convenient isolates for their introduction into the cultivated varieties.

MATERIAL AND METHODS

1 - Material

The isolates on which the study was effected were obtained from 22 species of Poaceae including 8 species of *Lolium* and 10 of *Festuca* (Table 1). *L.perenne* and *F.arundinacea* were the two species the most represented in the sampling, both by cultivated varieties and by plants of wild origin.

2 - Isolation methods

a) assessment of the presence of endophytes.

The mycelium is colored with Anilin blue 0.05%, especially on internal epiderm of the sheaths, according to the protocol of LATCH and CHRISTENSEN 1983.

b) isolation media

It was verified that antibiotics (penicillin 50 ppM + streptomycin 50 ppM) are without effect on growth of the endophyte. These antibiotics were then added to all isolation media.

Four different isolation media were compared: PDA, Malt-agar 2%, Yeast-Dextrose Agar (YDA) and the synthetic medium of MURASHIGE and SKOOG modified by addition of dextrose and casein. PDA and YDA generally gave the best results and the synthetic medium the lowest isolation rates. However, the results were dependent on the isolates.

c) sterilization of the samples

The pieces of tillers and leaf sheath were sterilized with bleach (12° Cl.). Comparison of different durations of sterilization were carried out, the optimal sterilization time was ten minutes.

The sterilization of seeds set particular problems. The protocol choosed consisted in a previous softening of the seeds in water for 36 hours, then elimination of the embryo and the ends of the seeds, then sterilization by alcool 70° (3 min) and bleach 12° Cl. (30 m.).

Table 1 - Origin of the isolates

1461	*Festuca arundinacea*	France, 46	Cahors	**
1781	*Festuca arundinacea*	France, 78	Grignon	**
1621	*Festuca arundinacea*	France, 62	Coulogne	**
1362	*Festuca arundinacea*	France, 36	St.Benoit-Du-Sault	**
1361	*Festuca arundinacea*	France, 36	St.Hilaire de Court	**
1861	*Festuca arundinacea*	France, 86	Oyré-les Communaux	**
1631	*Festuca arundinacea*	France, 63	?	**
Clarine	*Festuca arundinacea*	France	inconnu	*
1651	*Festuca arundinacea*	France, 65	Beaudéan	**
Epichloë3	*Festuca arundinacea*	inconnu	inconnu	*
Nui	*Lolium perenne*	Nouvelle Zélande	inconnu	*
11314	*Lolium perenne*	France, 13	Marseille	**
40128	Lolium perenne	Italie	Sardaigne	
11274	*Lolium perenne*	France	Cuzy	*
20088	*Lolium perenne*	Espagne	Grado	*
130003	*Lolium perenne*	Grèce	Dodomi	*
20118	*Lolium perenne*	Espagne	Sierra Nevada	*
217	*Lolium perenne*	France, 59	Hazebrouck	*
260	*Lolium perenne*	France, 86	Lussac-les-Chateaux	*
40137	*Lolium perenne*	Italie	inconnu	*
11313	*Lolium perenne*	France, 13	Marseille	*
11315	*Lolium perenne*	France, 13	Marseille	*
650003	*Festuca pratensis*	Norvège	inconnu	*
650005	*Festuca pratensis*	France	inconnu	*
650006	*Festuca pratensis*	inconnu	inconnu	*
40126	*Lolium multiflorum*	Italie	Cosenza	*
330009	*Lolium rigidum*	Tunisie	inconnu	*
11460	*Lolium rigidum*	France	Corse (Centuri port)	*
610005	*Lolium temulentum*	Portugal	inconnu	*
610007	*Lolium temulentum*	inconnu	inconnu	*
610008	*Lolium temulentum*	Tunisie	inconnu	*
620001	*Lolium remotum*	France	inconnu	*
630001	*Lolium persicum*	inconnu	inconnu	*
Epichloë2	*Poa nemoralis*	France, 63	Chassagne	**
640001	*Lolium subulatum*	Grèce	inconnu	*
163B	*Bromus ramosus*	France, 63	Montaigut le blanc	**
163R.N	*F. rubra nigrescens*	France, 63	St.Genès-Champanelle	**
Epichloë1	*Dactylis glomerata*	France, 63	Compains	**
163F1	*F. ovina filiformis*	France, 63	St.Genès-Champanelle	**
163H	*Festuca heterophylla*	France, 63	Chassagne	**
163A.L	*Festuca altissima*	France, 63	Compains	**
163G1	*Festuca gigantea*	France, 63	Montaigut le blanc	**
163G2	*Festuca gigantea*	France, 63	Chassagne	**
163G3	*Festuca gigantea*	France, 63	Dauzat sur Vodable	**
670001	*Festuca rubra*	France	inconnu	*
163A.R	*F. ovina arvernensis*	France, 63	St.Genès-Champanelle	**
700002(1)	*Poa. trivialis*	inconnu	inconnu	*
700002(2)	*Poa. trivialis*	inconnu	inconnu	*
020501	*Lolium canariense*	?	?	*

* cultivated ** wild

3 - Growth rate and incidence of temperature.

The growth rate was measured for all isolates.

a) the linear growth rate was evaluated at 24° C on all four culture media. The diameter of the thalli was measured avery four days during two months, on three repetitions of each isolate.

b) the weight growth was evaluated only on PDA (with 0.5% agar), but at five different temperatures: 12, 15, 20, 24 and 28° C. (three repetitions for each combination temperature x isolate).

4 - Macroscopic and microscopic morphology

a) microscopic morphology of the endophytic mycelium *in planta*.

The morphology of the endophytic mycelium in the sheaths or pith was described prior to isolation.

b) macroscopic description of thalli in pure culture.

The colour, structure, aspect of the thallus and the discoloration of the culture medium were described.

c) microscopic morphology: conidiophores and conidia.

Sporulation was assessed only at 24 ° and in some cases at 20°. When conidia were found, their lenghth and width were measured on 100 conidia, the average and standard error were calculated. The lenghth of conidiophores was also measured on 100 conidiophores.

5 - Response to benomyl

Eight concentrations of benomyl (1, 2.5, 5, 7.5, 10, 25, 50 and 100 ppM) were added to a PDA medium. A control without benomyl was also made. The weight growth was evaluated on three repetitions. This experiment was effected with only 10 isolates representative of the main groups.

6 - Isoenzymes

The mycelium was grown on Potato Dextrose Broth and freeze-dried. Extraction was carried out with Buffer tris at pH 7 containing ß-mercaptoethanol, EDTA, PVP, KCl and MgCl2.

Migration took place on starch gel 13%. A buffer Tris-citrate pH 7.2 was used for both making the gel and migration.

Four enzymes were searched: MDH (Malate Deshydrogenase), PGM (Phosphoglucomutase), PGI (Phosphoglucoisomerase), ACP (Acid Phosphatase).The enzymatic activities were located according to the procedures of Pasteur et al. (1987).

7 - Random Amplification of Polymorphic DNA (RAPD).

The mycelium was grown on Potato Dextrose Broth and freeze-dried. The extraction was effected according to the procedure of MOHAMMED (1994).

The PCR program consisted of a denaturation 5 min at 93°, then 40 cycles : denaturation 1min at 91°, annealing: 1 min at 36°, extension: 2 min at 70°. The last extension phase lasted 5 min.

17 primers were necessary to obtain a total of about 300 bands. A phenetic tree was drawn according to the UPGMA program with the distances of NEI and LI.

RESULTS

1) Isolations

About 40 isolates were obtained from the sheaths and stems of the different species (all the isolations succeeded). 6 isolates were obtained from the seeds out of 8 isolations tried. Four isolates were obtained from stromas of *Epichloë*.

About ten isolates of external origin were added to this collection.

2) Morphological variation

Differences were observed in the abundance, shape and degree of branching of the mycelium observed in sheaths and stems. For example, it was very twisted but poorly branched in most of the isolates from *L.perenne* and *F.arundinacea*. By contrast, it was highly branched for the three isolates from *F.gigantea*.

3) Sporulation, characteristics of the conidiophores and conidia

a) sporulation

The isolates from *L.multiflorum* and *F.pratensis*, and a majority of isolates from *L.perenne* did not sporulate at 20 or 24°C. The isolates from *F.arundinacea*, annual

Lolium, wild fescues and a minority of isolates from *L.perenne* sporulated at 24 °, as did the isolates of *Epichloë sp.*

b) shape and size of conidia

The isolates from annual *Lolium* (*L.rigidum*, *temulentum*, *remotum*, *persicum*, *subulatum* and *canariense*) appeared basically different from the other isolates as their conidia were rod-shaped, produced in large quantities by long, septate, conidiophores. The production of conidia by these conidiophores was continuous, and the spores were grouped in clusters at their tips. These conidia had a small size (4-7 microm. x 1-2 microm.). Indeed, these isolates seem to belong to genus *Cephalosporium* rather than *Acremonium*.

By contrast, the isolates from *F.arundinacea*, *L.perenne* (for those isolates sporulating at 24°),wild fescues, *Epichloe sp.* had kidney-shaped or navicular conidia carried by short, often non-septate conidiophores. The production of spores was not continuous, each conidiophore gave rise to only 1 to 3 conidia which generally took a position perpendicular to the conidiophore after they part from it.

Among these isolates with kidney-shaped spores, the isolates from *F.arundinacea* were different from the others by the large size of their conidia (8-13 x 2.5-4 microm. according to the isolates). The isolates from *L.perenne* and wild fescues and the isolates of *Epichloë* species had smaller conidia (4-7 x 2-3 microm.).

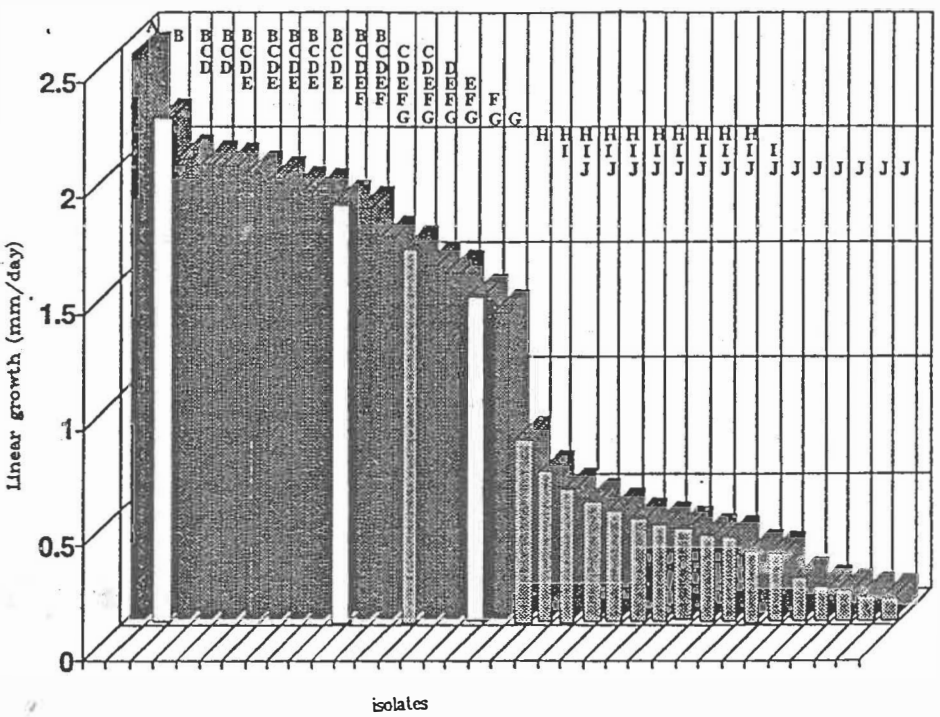
4) Growth of the isolates on four culture media

a) linear growth

The linear growth (mm./day) appeared highly variable, from 0.09 mm/day for isolate 40128 (*L.perenne*) to 2.45 mm/day for isolate 163F1 (*F.ovina filiformis*). Significative differences were also observed according to the media, the highest growth being observed on YDA, the poorest on the synthetic medium.

Two main groups clearly appeared (Fig.1): the isolates from *L.temulentum*, *L.perenne*, *F.pratensis* and *F.arundinacea* had a linear growth inferior to O.7 mm - a day (only one exception was noted), while the isolates from annual *Lolium* and wild fescues and the *Epichloë* isolates had a linear growth superior to 1.2 mm - a day. An additional result was that the isolates from *L.perenne* which sporulated at 24° had a higher growth than those which did not sporulate.

Figure 1 - Linear growth of *Acremonium* and *Epichloe* isolates



: *Epichloë sp*

: *Acremonium* isolates from *L.multiflorum, L.perenne, F.arundinacea, F.pratensis.*

: *Acremonium* isolates from annual *Lolium*, wild fescues, *Bromus ramosus* and *Poa trivialis.*

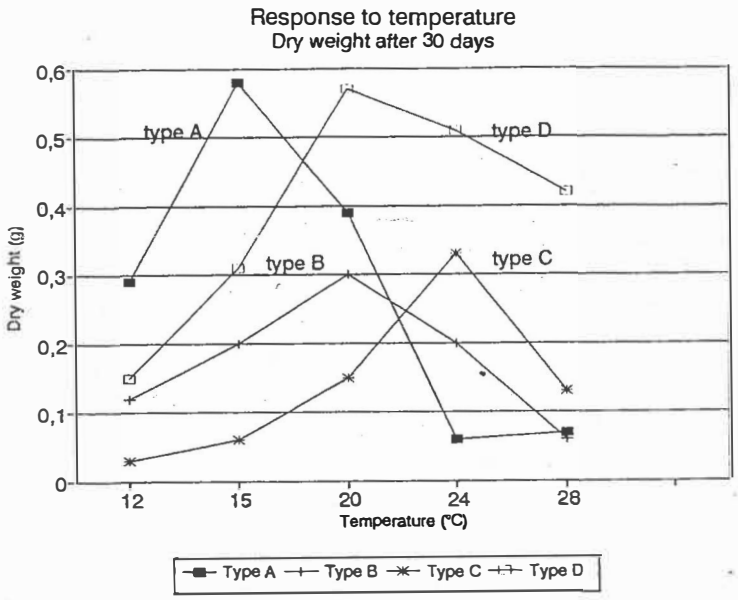
b) growth in weight

The results were fundamentally the same, although the separation between the two group was less clear-cut.

c) Influence of temperature

The responses to temperature appeared very diverse (Fig.2). However, intra-group variation for this factor appeared as important as inter-group variation.

Figure 2

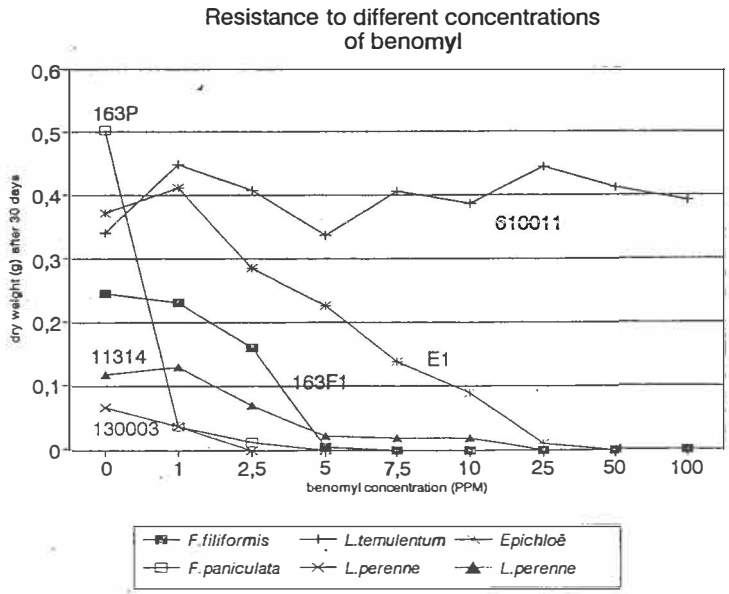


5) Response to benomyl

A large range of reactions was observed between the 10 isolates studied (Fig.3). On the one hand, the isolate n.610011 obtained from *L.temulentum* appeared completely insensitive even to high concentrations of benomyl. It will be necessary to verify if this behaviour is common to all isolates of this group (from annual *Lolium*) or if it is just the reaction of a particular isolate.

On the other hand, the isolate from *L.perenne* which did not sporulate at 24° and the one from *F.arundinacea* appeared highly sensitive to benomyl (no growth at 2.5 and 5 ppM of benomyl, respectively).

Figure 3



6) Isoenzymes

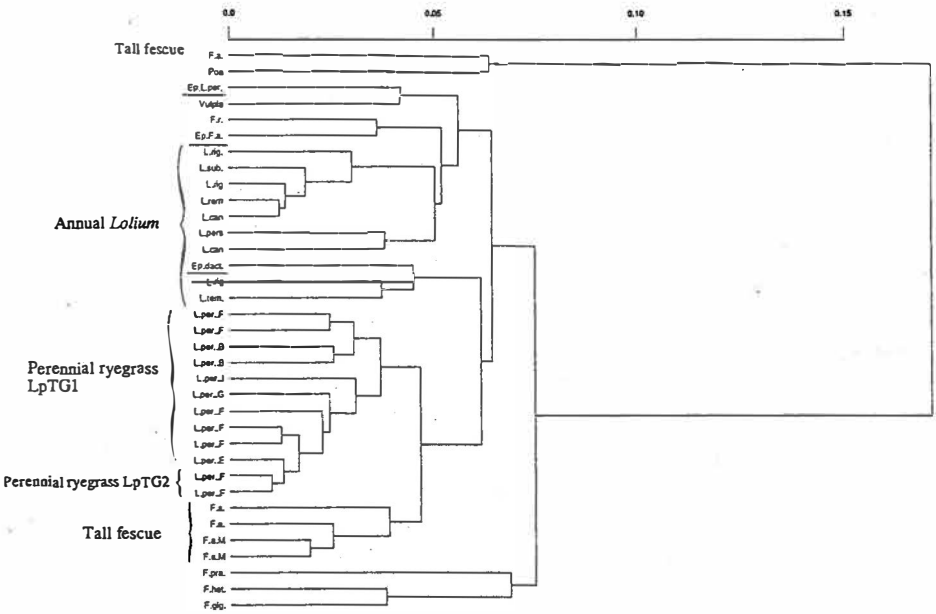
The results concerning the isoenzymes must be considered as preliminary. The work was mainly devoted to the isolates from *L.perenne* and *F.arundinacea*. The enzyme PGI (Phosphoglucose Isomerase) allowed discrimination between the two groups of isolates from *L.perenne*: three bands were observed with the isolates belonging to Group 1 (sporulating at 24°) and only 1 band for Group 2 (isolates not sporulating at 24°). With MDH, the isolates of group 1 also exhibited three bands while the pattern was more diverse for the isolates of group 2. The isolates from *F.arundinacea* had also 3 bands of MDH, but located at different positions.

The number of isolates from the annual species of *Lolium* which were analysed for their isozyme pattern was low and we do not know whether these isolates are homogeneous for this character.

7) R.A.P.D.

A UPGMA tree was established from 305 bands and tested by boot-strapping. The results obtained (Fig.4) showed that most of the inferior nodes (regrouping the isolates in elementary groups) were fairly reliable (probability > 10%). By contrast, the nodes of higher level (associating the groups with one another) had generally low probabilities.

Figure 4



Grass endophytes

U.P.G.M.A. tree

Distance of NEI and LI

309 bands R.A.P.D.

The isolates from *L.perenne* were all well regrouped within the tree. Most of them (10 /12) were belonging to the group which does not sporulate at 24°C. Only two sporulated at 24° C., they were classified very close to each other and not far from the isolates of the other group.

Among the 5 isolates from *F.arundinacea*, 4 were well regrouped while the 5th appeared completely different.

Nine isolates from annual *Lolium* species (with rod-shaped conidia) had been included in the analysis. 7 out of 9 were regrouped.

Only 3 isolates from wild fescues had been investigated. The isolates from *F.gigantea* and *F.heterophylla* were classified together, but far from an isolate from *F.rubra*. The three isolates of *Epichloë sp.* appeared very different from one another.

CONCLUSION

Most of the host plants that we have studied were represented in our sample by a small number of endophyte isolates, or even only one. Only two species: *Lolium perenne* and *Festuca arundinacea*, were represented by a high number of isolates of various origins, including both wild origins and cultivated vatieties. So, it is difficult to generalize the results obtained from one or two isolates from one host (especially as concerns the wild species of *Festuca*), and the results must be considered as preliminary.

The isolates studied could be distributed into 8 groups:

1) the isolates obtained from *Festuca pratensis* exhibited a very slow growth. These isolates did not sporulate at temperatures between 20 and 25°. Sporulation at lower temperatures was not attempted yet and so far we cannot confirm that these isolates belong to *Acremonium uncinatum*.

2) the same was true of the isolates obtained from Italian ryegrass, *Lolium multiflorum*, which were indeed difficult to conserve in pure culture.

3) all the isolates from *Festuca arundinacea* that were observed showed a slow growth, however sufficient to allow to study these isolates. Except rare exceptions, they sporulated at 20 - 25 °. The conidia were in all cases kidney-shaped, their size was high (8 - 13 microm.), the number of conidia produced on each conidiophore was low. All these isolates except one were characterized by three bands with MDH and one band with PGI at a constant RH. All these characteristics agree with species *Acremonium coenophialum*. Five of these isolates were studied in RAPD, four of them were regrouped in the tree.

4) the isolates from *Lolium perenne* could be clearly divided into two groups:

4a - the first one regrouped isolates with a medium growth rate, exhibiting a cottonous aspect in culture on PDA; these isolates sporulated at 20 - 24°, the conidia were kidney-shaped and small-sized (5 - 8 microm.). The isolates of this group showed three bands with MDH and also three bands with PGI. They could be successfully inoculated to seedlings of *Lolium perenne*.

This group coincides quite well with the species "LpTG-2" described by CHRISTENSEN et al (1993) and considered by SHARDL et al. (1994) as resulting from an interspecific hybridation between *Acremonium lolii* and *Epichloë typhina*.

4b - the second group regrouped a higher number of isolates exhibiting a slow growth and often a brain-like aspect on PDA. These strains did not sporulate at 20 - 24°, and our attempts to inoculate them on seedlings of *L.perenne* were unsuccessful. The strains of this group were characterized by a single band, in a constant position, with PGI and ACP. They were regrouped in a single cluster by RAPD. This group agrees with the species "LpTG1" or *Acremonium lolii sensu stricto* of CHRISTENSEN et al. (1993).

5) Twelve isolates were obtained from annual species of *Lolium* other than *L.multiflorum*. These included five autogamic species: *L.temulentum, L.remotum, L.subulatum, L.persicum, L.canariense*, and one allogamic: *L.rigidum*. These twelve isolates appeared remarkably homogenous. They exhibited a high grow rate whatever the medium and a cottonous aspect on PDA. They spoprulated profusely, the rod-shaped conidia carried in clusters by long conidiophores probably belong to a hitherto underscribed species of genus *Cephalosporium*.

The only isolate of the group which was tested for its response to benomyl appeared completely insensible to this fungicide.

These isolates were fairly well regrouped by RAPD markers. 6) The four isolates of *Epichloë sp.* included in our sampling were originating from four different hosts. The morphology of the ascospores and their pattern of disarticulation were not noted when the stromas were collected, so the exact taxonomical position of these isolates could not be specified. These four isolates had in common their high growth rate and their small, rare, kidney-shaped conidia. However, they appeared fairly variable by their isozyme and RAPD patterns.

7) The last group consisted of isolates from wild fescues. They shared with the *Epichloë* isolates a high growth rate and small kidney-shaped conidia. It is possible that a number of them belong to *Epichloë festucae* Leuchtmann, Schardl and Siegel.

Too few of these isolates were studied for their isozyme pattern to draw general conclusions. Only three of them were included in the RAPD studies, one strain from *F.gigantea* and *one* from *F.heterophylla* appeared close to each other and very far from one strain from *F.rubra.*

ACKNOWLEDGMENTS

We thank Pr. Paul NICOLAS from the University of Clermont-Ferrand II for his help in RAPD analysis.

REFERENCES

BACON C.W., PORTER J.K., ROBBINS J.D. & LUTTRELL E.S., 1977. **Epichloe typhina**.*Epichloë typhina* from toxic tall fescue grasses. **Appl. Environ. Microbiol.** 34, 576-581.

BREEN J.P., 1994. Acremonium endophyte interactions with enhanced plant resistance to insects. Ann.Rev.Entomol. 39, 401-423.

CHRISTENSEN M.J. & LATCH G.C.M., 1991. Variation among isolates of Acremonium endophytes (*A.coenophialum* and possibly *A.typhinum*) from tall fescue *Festuca arundinacea*. **Mycological Research** 95, 1123-1126.

CHRISTENSEN M.J., LATCH G.C.M. & TAPPER B.A., 1991. Variation within isolates of *Acremonium* endophytes from perelnnial rye-grasses. **Mycol.Res.** 95, 918-923.

CHRISTENSEN M.J., LEUCHTMANN A., ROWAN D.D. & TAPPER B.A. Taxonomy of *Acremonium* endophytes of tall fescue (*Festuca arundinacea*), meadow fescue (*F.pratensis*) and perennial ryegrass (*Lolium perenne*). **Mycol. Res.** 97, 1083-1092.

CLAY K., 1990. Fungal endophytes of grsses. **Annual Review of Ecology and Systematics.** 21, 275-297.

GAMS W., PETRINI O. & SCHMIDT D., 1990. *Acremonium uncinatum*, a new endophyte in *Festuca pratensis*. **Mycotaxon** 37, 67-71.

GUERIN D., 1898. Sur la présence d'un champignon dans l'Ivraie. **J.Bot.** 12, 230-238.

LATCH G.C.M., CHRISTENSEN M.J. & SAMUELS G.J., 1984. Five endophytes of *Lolium* and *Festuca* in New Zealand. **Mycotaxon** 20, 535-550.

LATCH G.C.M., CHRISTENSEN M.J. & HICKSON R.E., 1988. Endophytes of annual and hybrid ryegrasses. **New Zealand J. of Agric. Res.** 31, 57-63.

LEUCHTMANN A., 1994. Isozyme relationships of Acremonium endophytes from twelve *Festuca* species. **Mycol.Res.** 98, 25-33.

LEUCHTMANN A., SCHARDL C.L. & SIEGEL M.R., 1994. Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. **Mycologia** 86, 802-812.

LEUCHTMANN A. & CLAY K., 1990. Isozyme variation in the *Acremonium* / *Epichloë* fungal endophyte complex. **Phytopathology** 80, 10, 1133-1139.

LEWIS G.C. & DAY R.C., 1993. Growth of a perennial ryegrass genotype with and without infection by ryegrass endophyte and virus diseases. GBR, 14, 144-145.

MOHAMMED C. 1994. The detection and species identification of African *Armillaria*. in: Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification.A.SCHOTS, F.M.DEWEY & R.OLIDER, Ed., CAB International.

MORGAN-JONES G. & GAMS W., 1982. Notes on hyphomycetes. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloë typhina*, new taxa in one of the two new sections of *Acremonium*. **Mycotaxon** 15,311-318.

MORGAN-JONES G. & WHITE J.F.Jr., 1990. Endophyte-host associations in forage grasses. XIII- *Acremonium chilense*, an undescribed endophyte occuring in *Dactylis glomerata* in Chile. **Mycotaxon** 39, 441-454.

PASTEUR N., PASTEUR G., BONHOMME F., CATALAN J., BRITTON-DAVIDIAN J. 1987. Manuel Technique de Génétique par électrophorèse des protéines. Lavoisier, ed.,Paris

SAMPSON K., 1933. The systemic infection of grasses by *Epichloë typhina* (Pers.) Tul. **Trans. Br. mycol. Soc.** 18, 30-47.

SCHARDL C.L., LEUCHTMANN A., TSAI H.-F., COLLETT M.A., WATT D.M. & SCOTT D.B., 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloë typhina*. **Genetics** 136, 1307-1317.

SCHARDL C.L. & SIEGEL M.R., 1993. Molecular genetics of *Epichloë typhina* and *Acremonium coenophialum*. **Agriculture, Ecosystems and Environment** 44, 169-195.

VOGL A., 1898 Mehl und die anderen Mehlprodkte der Cerealien und Leguminosen. **Z.Nahrungam. Hyg.Warenkd.** 12, 25-29.

WEST C.P., IZEKOR E., TURNER K.E., ELMI A.A., 1993. Endophyte effects on growth and persistence of tall fescue along a water supply gradient. Agronomy Journal, 85, 264-270.

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., 1993. Endophyte - hosts associations in grasses. XIX- A systematic study of some sympatric species of **Epichloë** in England. **Mycologia** 85, 444-455.

WHITE J.F.Jr. & MORGAN-JONES G., 1987a. Endophyte-host associations in forage grasses. VII- *Acremonium chisosum*, a num species isolated from *Stipa eminens* in Texas. **Mycotaxon** 28, 179-189.

WHITE J.F.Jr. & MORGAN-JONES G., 1987b. Endophyte-host associations in forage grasses. X- *Acremonium starii*, a new species of endophyte. **Mycotaxon** 30, 117-125.

WHITE J.F.Jr., COLE G.T. & MORGAN-JONES G., 1987. Endophyte-host associations in forage grasses. VI- A new species of *Acremonium* isolated from *Festuca arizonica*. **Mycologia** 79, 148-152.

First results on the isozyme variation of isolates of *Acremonium* spp. from German pastures

Peter Dapprich[a], Karsten Krohn[b], and Volker H. Paul[a]

[a] Universität-Gesamthochschule-Paderborn, Fachbereich Landbau, Lübecker Ring 2, 59494 Soest

[b] Universität-Gesamthochschule-Paderborn, Fachbereich Chemie und Chemietechnik, Warburger Straße 100, 33098 Paderborn

Abstract

Plant samples of *Lolium perenne* were taken from four long term "low input" pastures in the area of Duisburg. From those pastures abnormal behaviour of the grazing young cattle was reported indicating in its symptomatology to the "ryegrass staggers syndrome". Analysis in our laboratories showed that those pastures had a high infection rate with endophytic fungi (DAPPRICH et al., 1995a). From 42 of those samples the endophytes were isolated and identified. In all cases of this examination the endophyt found was identified as *Acremonium lolii.*

Courtesy of Dr. Oldenburg two further field samples of endophyte infected *L. perenne* were at our disposal. Here *A. lolii* was likewise isolated. Furthermore *A. lolii* has been isolated from a seed sample of a Dutch *L. perenne* cultivar. All together 18 isolates of different origin of *A. lolii* isolated from *L. perenne* were multiplied by means of biotechnology and screened for possible differences in protein contents and isozyme patterns with the aid of polyacrylamidegel-electrophoresis (PAGE). So far the complete protein patterns as well as the patterns of the acid-phosphatase and the alpha-esterases have been compared. For the alpha-esterases two different substrates have been used which resulted in different banding patterns.

On account of these first results the *A. lolii* isolates of the Duisburg area can be divided into at least three groups. Compared with some foreign isolates both differences and similarities have been found. More detailed tests with different enzymes are necessary to

substantiate these preliminary test results. However from these examinations differences in physiology of *A. lolii* in grasses within one pasture could be seen to emerge.

Keywords: *Acremonium lolii*, *Lolium perenne*, German pastures, isozyme-variation

Introduction

Artificial infection methods with *Acremonium* species that colonise grasses still give great difficulties. Different trials have been attempted (LATCH & CHRISTENSEN, 1985; JOHNSON et al., 1986; RAVEL et al., 1994) whose low success rates were explained with the postulated high host specificity of *Acremonium* (LATCH & CHRISTENSEN, 1985; LEUCHTMANN, 1993). Besides this host specificity there are a number of reports about different combinations of *Acremonium* spp. and *Lolium perenne* or *Acremonium* spp. and *Festuca* spp. resulting in different amounts of alkaloid contents in the plant biomass (DOVALLE RIBEIRO, 1993). This concerns the total composition of the alkaloids as well as their concentration to each other. Those differences point to a variability of the host and/or the endophyte. Since grasses are cross pollinators they are the obvious choice for being responsible for the genetical and physiological variability. In *Acremonium* species isolated from *Festuca* a physiological variability was described by LEUCHTMANN and CLAY (1990). They found a coherence between the variation of the enzyme patterns of *Acremonium* isolates of *Festuca* separated by means of starchgel electrophoresis and alkaloid production in host biomass. Comparative analysis of the enzyme patterns showed a new *Acremonium* group, which did not resemble any species described so far (CLAY & LEUCHTMANN, 1989; CHRISTENSEN & LATCH, 1991; LEUCHTMANN, 1994). From the tested *A. lolii* isolates from *L. perenne* so far only one phenotype has been reported. In the work at hand first results of the isozyme analysis of different *Acremonium* isolates through polyacrylamidegel-electrophoresis (PAGE) are presented. Different isolates from neighbouring pastures as well as of different national and international origin were compared.

Materials and Methods

Samples from pastures in the Duisburg-area were taken as described earlier (DAPPRICH et al., 1995a). The isolation of the endophytic fungi was performed in modification to the method of LATCH & CHRISTENSEN (1985) according to DAPPRICH et al. (1995a). Furthermore *A. lolii*-isolates were obtained from two pasture-samples kindly provided to us by Dr. Oldenburg, FAL, Braunschweig, one Dutch cultivar and cultivars from the United States and Australia. In addition isolates were supplied by Dr Grand-Ravel (France) and Dr Garthwaite (New Zealand). Other isolates originated from the Centraalbureau voor Schimmelcultures (CBS, Baarn, NL). The origin of all endophytes used in this work are summarised in Table 1.

Origin of plant material	code
long term „low input" pasture (Duisburg area, Northrhein Westfalia)	1/94, 10/94, 16/94, 22/94 28/94, 37/94, 38/94
long term pastures (Germany) (kindly provided by Dr Oldenburg, FAL)	82/83 82/89
Dutch seed sample	K +
French isolate (kindly provided by Dr Grand-Ravel)	F-573
North American isolate (cv. Repell)	R-USA
New Zealand isolate (kindly provided by Dr Garthwaite)	Lp 19/188 S
cultures from the Centraalbureau voor Schimmelcultures (CBS, Baarn, NL)	CBS 229-84 CBS 232-84

Table 1 *Acremonium lolii* isolates from *Lolium perenne* used for protein analysis

Biomass production of the endophytic fungi was carried out in 500 ml Erlenmeyer-flasks in 200 ml potato-dextrose-broth (PDB) sealed with an aluminium cap and parafilm. As inoculum we used about 1 cm^2 of a well growing culture on potato-dextrose-agar (PDA) homogenised in 40 ml PDB in a warring blender under sterile conditions. 10 ml of this suspension was added to each flasks. The incubation occurred on an orbital shaker at 120

rpm and room temperature. Depending on the growth of the isolates 4 - 6 weeks later the biomass was separated from the medium by vacuum filtration and freeze dried. The dried mycelium was powdered in liquid nitrogen and water soluble proteins were extracted out of 100 mg of each isolate. As an extraction buffer we used one ml of a 0.1 M tris and 0.5 M sucrose solution with a pH of 7.0. After shaking 30 sec. in 1.5 ml Eppendorf reaction tubes at room temperature (vortex) samples were placed in a frozen (-20°C) rotor of a Sigma 202-C centrifuge immediately. Mycelium and non soluble particles were sedimented by 10 minutes of centrifugation at 10.000 rpm. The protein containing supernatant was used for electrophoresis

a) directly in varying quantities

b) in volumes of 10 µl after adjusting the protein contents to 5 mg/ml

Estimation of protein contents was carried out using the bicinchoninic acid-method published by Sigma (Sigma procedure No. TPRO-562). Acetone was used for protein concentration following the method of HAMES & RICKWOOD (1990).

For the protein separation we used continuous gradient gels from 8 - 18 % polyacrylamide with at least 1 cm of an 8 % stacking gel. The gel-preparation and electrophoresis followed the instructions of HAMES & RICKWOOD (1990). Separation occurred over night in native conditions with 14 mA set and variable voltage.

For total protein detection we used Coomassie Blue R 250 according to HAMES & RICKWOOD (1990), isozyme identification followed the methods described by BURHENNE (1992) for acid phosphatase and alpha esterases.

Results and Discussion

The use of PAGE for the separation of water soluble endophytic proteins appeared to be very successful. Though the making of polyacrylamide-gels is more difficult and more time consuming than using starch-gels it results in a more sharp and better distinguishable banding pattern (Plate 1). Furthermore using a continuous gradient of polyacrylamide concentration in the gels depending on the molecular size of proteins to be separated it results in even better separation and better bandings than using homogenous gels (Plate 2).

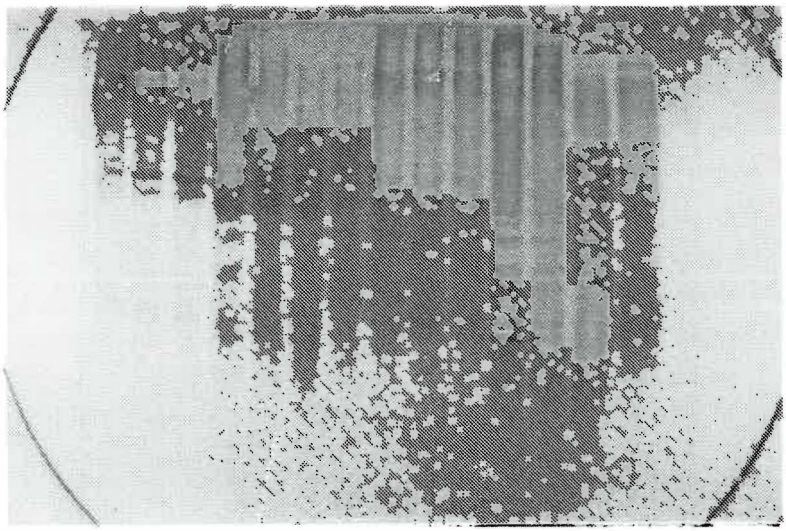


Plate 1 Analysis of water soluble proteins from endophytic fungi of the genus *Acremonium* using a 12 % homogenous PAGE and Coomassie Blue R 250 as a staining agent

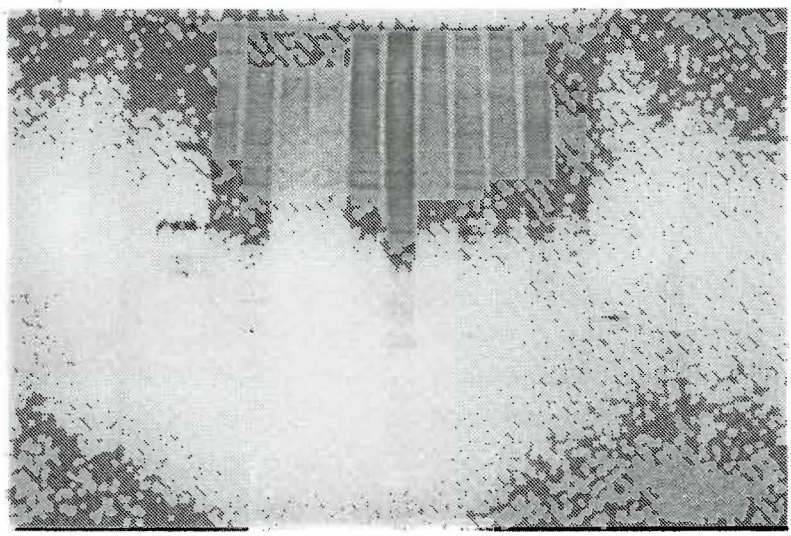


Plate 2 Analysis of water soluble proteins from endophytic fungi of the genus Acremonium using an 8 - 15 % continuous gradient PAGE and Coomassie Blue R 250 as a staining agent

Our results indicate that the isolates from the Duisburg area can be grouped into at least three sections. Section 1) with the isolate 1/94, section 2) with the isolate 22/94, section 3) with the isolates 10/94, 16/94 and 38/94 (Table 2). With all of these isolates from section 3) we found identical banding patterns with the *A. lolii*-isolate from the *L. perenne* cultivar Repell obtained from the USA. The protein patterns from the New Zealand isolate and from CBS232-84 also appeared to be identical, yet different to group 1 - 3, so they were preliminary sectioned into group 4.
The other Duisburg isolates, 28/94 and 37/94 could not yet be classified in one of these or some new groups.

group 1	1/94
group 2	22/94
groep 3	10/94, 16/94, 38/94, R-USA
group 4	Lp 19/188 S, CBS 232-84
not classified	28/94, 37/94, K +, 82/83, 82/89, CBS 229-84, F-573

Table 2 Preliminary grouping of the Acremonim lolii isolates tested after isoenzyme analysis

The two isolates from Braunschweig appeared to be different from each other, too, and there was no similarity with the Duisburg, Dutch, French, Australian or New Zealand-isolates, either. In addition we could not detect any similarity between these international isolates.
Differences or similarities were difficult to detect when testing with acid-phosphatase. Protein bands were very intense and diffuse, making it quite often impossible to identify the exact position of the protein associated with the reaction. Yet, there were no contradictions with regard to the results obtained with alpha-esterases.
An interesting effect could be observed when testing for alpha-esterases with different substrates. One CBS-isolate (CBS 232-84) and the French isolate showed identical patterns with the original substrate 1-Naphthylacetate. Tested with 2-Naphthylacetate as a substrate CBS 232-84 revealed obvious and reproducible differences by showing an additional band while the French did not (Plate 3 lane 4 and plate 4 lane 3). A similar situation we found for 38/94 and CBS 229-84 and for both the CBS-isolates.

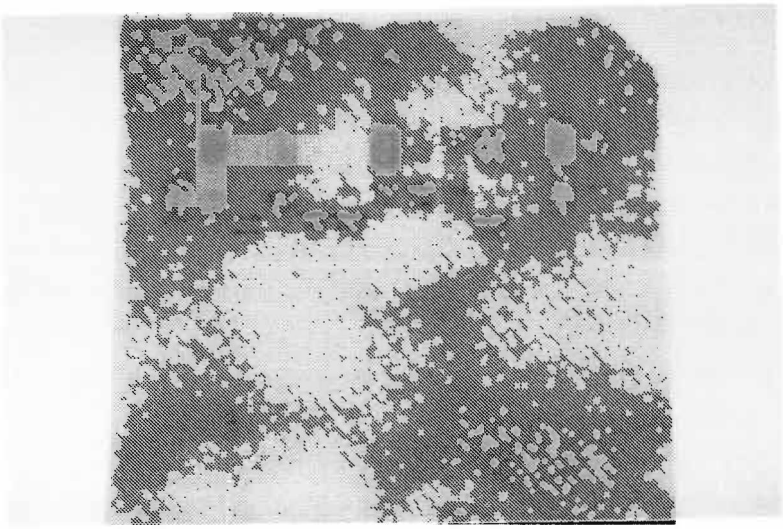


Plate 3 Protein analysis using an 8 - 15 % continuous gradient PAGE stained for alpha esterases using 1-Naphthylacetate as a substrate

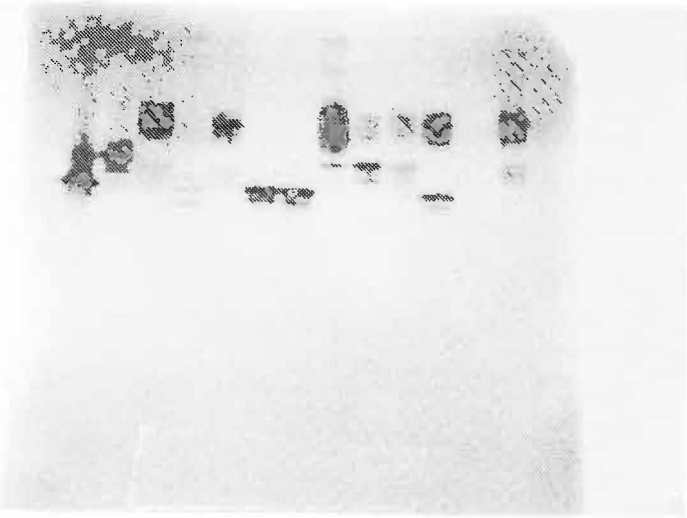


Plate 4 Protein analysis using an 8 - 15 % continuous gradient PAGE stained for alpha esterases using 2-Naphthylacetate as a substrate

The isozyme variation for the two tested enzymes acid phosphatase and alpha-esterases as well as the comparison of the total protein patterns detected with Coomassie Blue R 250 indicates that there is quite some difference in the physiological activity of *A. lolii*-isolates. This differences can be found in one single and in neighbouring pastures as well as in isolates from different countries or geographical origin. Based upon isozyme variation it seems possible to establish physiologically different groups of *A. lolii*-isolates for various purposes. According to LEUCHTMANN (1994) these isozyme-variations can be related not only to different potentials in alkaloid-biosynthesis but to differences based on DNA level.

With more detailed information obtained from isozyme-analysis it might be possible to define new species of *Acremonium*-endophytes not only in *Festuca* spp. and *Lolium* spp but in various grasses. Thus, such results can support the existing morphology-based taxonomic system as proposed by MICALES et al. (1986). Furthermore it might be possible to predict a potential harm to grazing livestock or possible benefit to the grass plants on basis of isozyme analysis. Given enough information on endophytes with different biochemical activities it might be possible to create even better specific mixtures of grasses for various purposes in grassland, pastures and turf than it is possible now, by selection of endophyte free cultivars.

These first results demonstrate the necessity of further investigation on the biochemical connections between grass and endophytes. More knowledge on the diversity of endophyte-physiology might elucidate the host specificity of most endophytes. Then this information might help to understand the reasons why artificial infection of grasses with endophytes still is a major problem.

Acknowledgements

We are grateful to the Federal Ministry of Food, Agriculture and Forestry (Bundesministerium für Ernährung, Landwirtschaft und Forsten (BML)), and the Society for Sponsering of Private German Plant Breeding (Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e. V. (GFP)) for their financial support.

References

Burhenne, S. (1992). Zur Charakterisierung und zur Biochemie der Blattfleckenerreger *Drechslera siccans* (Drechsl.) Schoem. und *Drechslera andersenii* (Scharif) Lam im Hinblick auf die Krankheitsresistenz von Lolium perenne. Dissertation des Fachbereich Chemie und Chemietechnik, Universität-GH-Paderborn

Christensen, M.J. and Latch, G.C.M. (1991). Variation among isolates of *Acremonium* endophytes (*A. coenophialum* and possibly *A. typhinum*) from toxic tall fescue (*Festuca arundinacea*). Mycol.Res. 95(9): 1123-1126

Clay and Leuchtmann, A. (1989). Infection of woodland grasses by fungal endophytes. Mycologia 81(5): 805-811

Dapprich, P., Paul, V.H., and Krohn, K. (1995). Incidence of *Acremonium* endophytes in selected German pastures and the contents of alkaloids in *Lolium perenne*. in Press

DoValle Ribeiro, M.A.M. (1993). Transmission and survival of *Acremonium* and the implication for grass breeding. Agriculture, Ecosystems and Environment, 44: 195-214

Hames, B.D. and Rickwood, D. (1990). Gel electrophoresis of proteins. Oxford University Press, Oxford, New York, Tokyo

Johnson, M.C., Bush, L.P., and Siegel, M.R. (1986). Infection of tall fescue with *Acremonium coenophialum* by means of callus culture. Plant Disease, 70(5): 380-382

Latch, G.C.M. and Christensen, M.J. (1985). Artificial infection of grasses with endophytes. Ann.appl.Biol., 107: 17-24

Leuchtmann, A. (1994). Isozyme relationships of *Acremonium* endophytes from twelve *Festuca* species. Mycol.Res., 98(1): 25-33

Leuchtmann, A. and Clay, K. (1990). Isozyme variation in the *Acremonium/Epichloe* fungal endophyte complex. Phytopathology, 80(10): 1133-1139

Leuchtmann, A. and Clay, K. (1993). Nonreciprocal compatibility between *Epichloë* and four host grasses. Mycologia, 85: 157-163

Micales, J.A., Bonde, M.R., and Peterson, G.L. (1986). The use of isozyme analysis in fungal taxanomy and genetics. Mycotaxon, 27: 405-449

Ravel, C., Wartelle, D., and Charmet, G. (1994). Artificial infection of tillers from perennial ryegrass mature plants with *Acremonium* endophytes. IOBC wprs Bulletin, 17(1): 123-125

Molecular analysis of the pathogen response of ryegrasses upon infection with crown rust

Adams E.[1], Van Bockstaele E.[1], Depicker A.[2] & De Loose M.[1]

[1] Rijksstation voor plantenveredeling, Burg. Van Gansberghelaan 109 B-9820 Merelbeke
[2] Laboratorium voor Moleculaire Genetica, K. L. Ledeganckstraat 35, B-9000 Gent

Introduction

Ryegrasses (*Lolium* sp.) are cross-pollinating species, used for pastures and recreation grass fields. The aim of this work is to obtain less susceptible ryegrass plants for *Puccinia coronata* by developing a more efficient selection strategy for breeding programs. The biotrophic fungus *Puccinia coronata* causes the crown rust disease, an important disease for ryegrasses (Potter, 1987).

Two approaches will be used. Plant genes which are specifically expressed upon infection will be identified and characterized. These genes can be used to enhance the resistance via overexpression of these genes upon appropriate inductive signals. Another approach will be the identification of molecular markers which are linked with resistance genes by bulked segregant analysis (BSA), allowing an indirect selection during breeding.

Materials and methods

The EMBL-database was screened for sequences encoding pathogenesis related (PR) proteins in higher plants. These were classified and aligned. Based on the most conserved DNA sequences, primers were designed for PCR and RT-PCR reaction.

For the bulked segregant analysis (BSA) (Michelmore *et al.*, 1991), both susceptible and tolerant Italian ryegrass clones were selected after a severe crown rust infection in the field. The tolerant clones came from the cultivar Axis. F_1 populations were produced from pair crosses between tolerant and susceptible plants, which were crossed between each other to produce the F_2 populations. The artificial infection was essentially carried out as described by Birckenstaedt *et al.* 1992. The AFLP® marker system (Zabeau & Vos, 1993) was optimized on the parent plant clones for the BSA analysis.

Results

A search of the EMBL-database for PR sequences revealed hundreds of PR sequences from different plant species. Figure 1 gives an overview of the approach. The PR sequences were classified in groups according to their homology. The DNA sequences encoding PR proteins from class 1 and from class 5 were aligned. These two alignments revealed different conserved regions. From these regions, sequences were selected for the design of a set of primers. After PCR reaction with these primers on genomic Italian ryegrass DNA as template, several DNA fragments were observed. None of the fragments hybridized with probes from PR1 and PR5 sequences from tobacco. To enhance the probability to amplify a PR coding sequence by PCR, the same approach was applied on reverse transcribed mRNA (RT-PCR). The mRNA was obtained from plants treated with salicylic acid, an artificial inducer of pathogen responses (Raskin, 1992). Three fragments amplified with the PR1 primers were cloned and sequenced. A homology search in the EMBL database revealed indeed a significant homology of only one of the clones with PR1 sequences from different species.

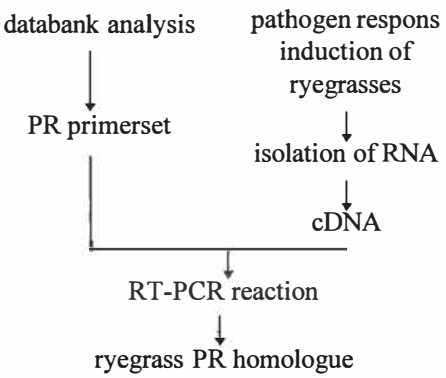


Fig. 1 : isolation of PR homologues

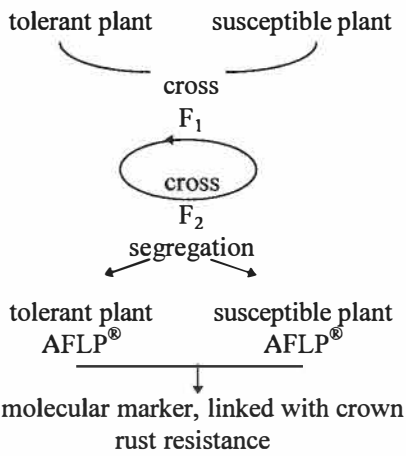


Fig. 2 : bulked segregant analysis

Conclusions

Firstly, by applying specific primers in an RT-PCR reaction, a PR1 specific ryegrass DNA sequence has been cloned. This will allow us to characterize the PR1 gene family and its gene expression patterns in different environmental conditions. Secondly a segregating population for crown rust tolerance was obtained which will allow to apply the BSA technique for the detection of a molecular marker, linked to a resistance locus or loci..

References

Birckenstaedt, E., Hardtke, S., Lorenz, E., and Paul, V.H.(1992). *Cereal Rusts and Powdery Mildews Bulletin* **20**, 14-25.

Michelmore, R.W., Paran, I., and Kesseli, R.V.(1991). *PNAS. USA* **88**, 9828-9832.

Potter, L.R. (1987). *Pl.Pathology* **36**, 455-461.

Raskin,I.(1992).*Ann.Rev.Pl.Physiol.Pl.Mol.Biol.***43**, 439-463.

Zabeau, M. & Vos, P. (1993). *European Patent Application* **EP 534858 A1**.

E.A. was supported by a grant from the "Vlaams instituut voor de bevordering van het wetenschappelijk-technologisch onderzoek in de industrie (IWT)"

Evaluation of susceptibility to leaf pathogens of meadow fescue forms regenerated from calli selected on the metabolites of *Drechslera dictyoides* (Drechsl.) Shoem. and *Bipolaris sorokiniana* (Sacc.) Shoem.

AGNIESZKA PŁAŻEK

Plant Breeding and Acclimatization Institute, Department of Fodder Crops, Kraków, Poland

ABSTRACT

The aim of this study was the evaluation of the resistance of *Festuca pratensis* (Huds.) forms obtained from calli selected on the metabolites of *Drechslera dictyoides* and *Bipolaris sorokiniana* to these pathogens in the field conditions. The progeny S_1 of four genotypes regenerated of calli selected on metabolites of *B.sorokiniana* were inoculated with spores and mycelium of this pathogen in greenhouse conditions. Field resistance of all forms to leaf spot pathogens was estimated on the basis of macro- and microscope observation. The following species were identified as the most common ones: *D. dictyoides, D. avenae, D. siccans* and *D. noblae.* All forms (10 genotypes) obtained from calli selected on the *D. dictyoides* metabolites were extensively infected with this pathogen. Only two forms of 11 genotypes obtained from calli selected on the *B.sorokiniana* metabolites the occurence of this fungus was observed. The progeny S_1 of four chosen genotypes showed higher resistance to artificial inoculation with spores and mycelium of *B. sorokiniana* than control plants of the same meadow fescue cultivar. The method using plant tissue selection on medium with fungus metabolites is not effective in the breeding of forms resisting to *D. dictyoides.* These works are promising in the breeding to *B. sorokiniana.*

Keywords: *Bipolaris sorokiniana, Drechslera dictyoides,* meadow fescue, metabolites, resistance

INTRODUCTION

The most common pathogens which cause leaf spot on *Festuca pratensis* and other fodder grasses are the fungi of *Drechslera sp.* The appearance of *Drechslera dictyoides* decreases the green mass yield in significant degree. The fungi of *Drechslera sp.* are worthy of special notice because of their secondary metabolites which can exhibit various biological activity: antibiotic, phytotoxic and even zootoxic (f.e. sterigmatocystin) properties (Shotwell and Ellis 1976, Durbin 1981, Engstrom et al. 1993). One of the most important species which can produce the toxic compounds is *Bipolaris sorokiniana.*

The subject of these studies was the evaluation of resistance of *Festuca pratensis* forms obtained from calli selected on the metabolites of *Drechslera dictyoides* and *Bipolaris sorokiniana* to these pathogens in the field conditions. Also the resistance of progeny S_1 obtained of four genotypes regenerated on *B.sorokiniana* metabolites was estimated after artificial inoculation with spores and mixed mycelium of this pathogen.

MATERIAL AND METHODS

The method of the selection of calli on the above mentioned fungi metabolites was presented in the earlier paper (Płażek 1994). This method used the double-layer technique described by Lepoivre et al. (1986). Regenerated plants which did not demonstrate disease symptoms after artificial inoculation with spore suspension in greenhouse conditions were planted in the field. 10 genotypes were regenerated of calli selected on metabolites of *D. dictyoides* and 11 genotypes of calli selected on metabolites of *B. sorokiniana*. All genotypes were obtained from calli of meadow fescue, Polish cultivar Skrzeszowicka.

Field resistance of all forms to leaf pathogens was estimated on the basis of macro- and microscope observations. From each examined plant ten leaves were randomly cut off and put on plastic dishes onto wet filter paper. The dishes were lit with NUV light in order to stimulate sporulation of the pathogens occuring on the leaves.

Four fescue genotypes obtained from calli put on the medium containing *Bipolaris sorokiniana* metabolites were self-pollinated. Collected seeds (S_1) and seeds of control plants of Skrzeszowicka cultivar were sawed into pots in the greenhouse. The seven-week seedlings were infected with spores and mixed mycelium of *Bipolaris sorokiniana*. After

ten days the infection degree was scored on a scale 0-5 where 0 means a plant without disease symptoms and 5 means a whole plant covered with spots. Disease coefficient was calculated for the progenies S_1 seperately of each genotype after a formula:

$W = \Sigma (k \times n) / N$ where symbols mean:

k - infection degree

n - number of plants of given degree

N - total number of infected seedlings

RESULTS

In the field the occurence of powdery mildew was not noticed while symptoms of rust were recorded only on a few forms of fescue. These symptoms could be described as trace ones expect of plants of one genotype on which signs of very strong infection with this pathogen were observed. On all studied plants many leaf spots were observed.

By means of microscope the following species were identified as the most common ones: *Drechslera dictyoides* (on 17% of examinated leaves), *D. siccans* (14,5%), *D. nobłae* (5,4%) and *D. avenae* (3,6%). All regenerated plants obtained from calli selected on the *D. dictyoides* metabolites were extensively infected with this pathogen, although only on two genotypes from 11 genotypes obtained from calli selected on the *B. sorokiniana* metabolites the occurence of this fungus was observed .

Table 1. Percentage of studied genotypes S_1 and control plants of meadow fescue cultivar Skrzeszowicka in given infection degree and their disease coefficients after artificial inoculation with spores and mixed mycelium of *Bipolaris sorokiniana.* Seedlings were scored on a scale 0-5.

No of geno type	No of infected plants	0	1	2	3	4	Disease coefficient
I	221	32,6	41,2	24,4	1,8	-	0,95
II	60	28,3	55,0	15,0	1,6	-	0,9
III	232	55,6	35,8	6,5	2,2	-	0,46
IV	186	51,6	36,6	11,8	0,0	-	0,6
Control	254	3,1	30,3	37,0	24,8	4,7	1,98

Table 1. demonstrates the infection degree of plants inoculated with spores and mycelium of *B.sorokiniana*. 45% of 699 inoculated seedlings were without disease signs and only 1% of plantlets were estimated on a scale as 3. The control (254 plants) was more susceptible to artificial infection than mentioned progeny S_1. Only 3% of them were without disease symptoms and 5% of seedlings were covered with spots in degree of 4. In the same table disease coefficients calculated seperately for each studied genotype are presented.

DISCUSSION

Microscope studies of leaves cut off plants regenerated in vitro showed that all forms obtained from calli selected on medium with metabolites of *D. dictyoides* were infected with spores of this fungus and only on two forms of 11 genotypes regenerated from calli selected on metabolites of *B. sorokiniana* appearance of its spores was noticed. The experiments using plant tissue selection in vitro on the fungus metabolites can be promising in case of these fungi which produce toxins for example *Helminthosporium victoriae* (Rines and Luke 1985), *Helminthosporium sativum* (Chawla and Wenzel 1987), *Drechslera teres* (Hunold et al. 1992).

Sexual progenies of regenerants usually are tested for its resistance to given pathogen (Wenzel and Foroughi-Wehr 1990, Posselt and Altpeter 1993). These authors did not obtain significant differences between resistance reactions of studied regenerant plants and their progeny. However they mention that plant cell cultured in vitro can express genetic variability and can be regenerated into plants with higher disease resistance. The experiment described in this paper indicated that the progenies obtained after self - polination are more resistant to infection with spores and mycelium of *B. sorokiniana* than control plants of the same cultivar.

D. dictyoides is not considered as toxin-producing pathogen. The resistance breeding to this fungus is very important because it appearences commonly on meadow fescue. In this case somaclonal variation occuring in tissue culture could be helpful or we should search for other way for example to find out the physiological or biochemical mechanisms which occure in plants infected with this pathogen.

CONCLUSIONS

The method of the breeding of calli on the medium with fungus metabolites is not effective in the works selecting fescue forms resisting to *Drechslera dictyoides*. However, these works are promising in the resistance breeding to *Bipolaris sorokiniana*.

REFERENCES

Chawla, H.S., Wenzel, G. 1987: In vitro selection of barley and wheat for resistance against *Helminthosporium sativum*, Theor. Appl. Genet, 74: 841-845

Durbin, R.D. (red) :1981, Toxins in plant disease. Academic Press, New York-London-Toronto-Sydney-San Francisco.

Engstrom, K., Brishammar, S., Svensson, C., Bengtsson, M., Andersson, R., 1993: Antraquinones from some *Drechslera* species and *Bipolaris sorokiniana.* Mycol. Res. 97 (3): 381-384.

Hunold, R., Hartleb, H., Afanasenko, O.S., 1992: Resistance against *Drechslera teres* (Sacc.) Shoem. in progenies of in vitro selected callus derived plants of barley (*Hordeum vulgare* L.), J. Phytopathology 135: 89-98.

Lepoivre, P., Viseur, J., Duhem, K., Carels, N., 1986, Double-layer culture technique as a tool for the selection of calluses resistant to toxic material from plant pathogenic fungi. J. Semal (Ed) Somaclonal variation and crop improvement, Martinus Nijhoff Publ.: 45-53.

Płażek, A. 1994, *In vitro* selection of *Festuca pratensis* (Huds.) callus on the metabolites of *Drechslera sp.* pathogens. Proceedings of the 19th Fodder Crops Section Meeting held in Brugge, Belgium, 5-8 October 1994: 183-184.

Posselt , U.K., Altpeter, A. 1994: In vitro selection for snow mould resistance in perennial ryegrass. Proceedings of International Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf. Paderborn (Germany) 4-6 October 1993 in IOBC/WPRS Bulletin Vol.17(1) 1994: 225-227.

Resistance Breeding

A NEW SCREENING TEST FOR RESISTANCE OF TURF GRASSES TO THE RED THREAD DISEASE (*LAETISARIA FUCIFORMIS* Mc Alp., Burdsall): PRELIMINARY RESULTS

GONDRAN, J.

INRA, PATHOLOGIE, STATION D'AMELIORATION DES PLANTES FOURRAGERES, 86600, LUSIGNAN, FRANCE

ABSTRACT

The red thread disease caused by *Laetisaria fuciformis* is one of the main disease of turf grasses in temperate regions of the world. Breeders cannot select individual resistant plants because plants growing apart are seldom diseased in natural infections. In this site plants sown at high density can be sick, but these infections are often heterogeneous and the varietal resistances cannot be distinguished. Moreover the plants are entangled and cannot be easily selected.

We have defined an artificial infection method of turf grasses.This method used high doses of barley grain inoculum applied on spaced plants (4 X 6 cm apart) in a poor soil (sand and earth, ¾ and ¼ by volume respectively, without fertilizer and limited watering). Our experiment demonstrated that the resistance of four varieties of *Lolium perenne* and *Festuca rubra* ranked as in field conditions : 'Limage' was more resistant than 'Elka' and `Rubina' was more resistant than 'Ensylva'. Two procedures proved efficient to distinguish the resistance of the four varieties :

1)-isolation of the fungus from a red fescue diseased plant and application of dry inoculum (69% w/w water) at 92 g/dm².

2)-Isolation of the fungus from a perennial ryegrass diseased plant and application of dry inoculum (7% w/w water) at 3.5 g/dm². It is noticeable that when inoculum is prepared with this isolate and applied humid at the doses 23 g/dm², 46 g/dm², 92 g/dm², no difference between varieties can be observed.

Before recommending a current use of this method it would be necessary to control the origin and the pathogenicity of fungal isolates, to define more accurately the inoculum doses and to validate it through multiple experiments and through multiple locations.

KEY WORDS : RED THREAD DISEASE, TURF GRASSES, BREEDING TEST FOR DISEASE RESISTANCE

INTRODUCTION

The acreage of turf grass lawns is increasing every year in temperates regions by the development of towns, motorway verges, playing fields and domestic lawns. In these regions, the red thread disease (*Laetisaria fuciformis)* and the pink patch disease *(Limonomyces roseipellis*), appear to be the most important fungal injuries (GONDRAN and COURTILLOT 1994). *L. fuciformis* seems more frequent than *L. roseipellis* (O'NEIL and MURRAY 1985). In artificial infections we have observed (GONDRAN, 1995) that two isolates of *L. fuciformis* were more pathogenic than two isolates of *L. roseipellis*. So it is likely that the red thread disease is more dangerous than the pink patch disease.

It is possible to control this disease by using nitrogen fertilizers, sowing resistant species and cultivars and spraying fungicides (SMITH *et al.*, 1989). But these *controls* are often ineffective because soils have too much sand to prevent waterlogging and to get successful sowings. These unfertile soils increase the infection and invalid the *controls*. To improve this situation, we think to rise the resistance level of the varieties. But breeders depend on natural infections which are characterized by a high variability masking varietal differences. Moreover plants sown in high density are entangled and cannot be easily selected whereas low density plots are seldom infected.

We defined an artificial infection of spaced plants, then we carried out an experiment to reveal the variety disease resistance. Possible improvements will be discussed.

MATERIALS AND METHODS

1)-The artificial infection

The plants were grown in 15x45x15 cm metallic trays, which we filled up with a sand and soil 3:1 v/v, without fertilizer (0.5 nitrogen p. 1000) at a density of 20 plants per tray.The space between plants was 4 cm in a row with rows 6 cm apart. Each plant was separated from the next by a piece of polyvinyl pipe 3 cm diameter and 5 cm hight. After 4 months in a non heated greenhouse the plant occupied all the space inside the pipe and the plants were ready to be infected.

The fungus was maintained in test tubes of potato sucrose agar medium at 5°C. French bottles with barley grains were autoclaved and later seeded with the fungus. After 45 days at 20°C the fungus fully colonized the grains. These grains can be spread immediately on the plants and as they contained 69% w/w water we called them **humid inoculum**. If a **dry inoculum** was wanted (7% w/w water), the fresh barley grains colonized by the fungus were spread on the surface of wire trays during one month at 20°C (Both inoculums were weighed in order to use them as doses in g/dm²).

From the begin of May to the end of June the plants growing in the trays were moved outside the greenhouse to experience the natural conditions of the oceanic climate of Lusignan (west center of France, see table 1). It is necessary to have this alternance of temperatures every 24 hours to obtain good symptoms (BAHUON, 1986).

Table 1 : Means during 30 years (1965 - 1994) of some climatic data in Lusignan.

Months	May	June	July	August	September	October
Rains (mm)	81.1	51.5	46.5	53.8	69.0	74.8
Night minimal temperature	8.03	11.03	13.05	12.71	10.91	7.77
Day maximal temperature	17.85	21.69	24.70	24.12	21.56	16.83

Before the infection the plants were cut just at the top of the pipe, i.e. 2.5 cm above the soil surface. The inoculum was then poured above the cut plants. In case of large dose of inoculum it was necessary to press it inside the hole of the pipe, because the infected grains outside the pipe were lost for contamination. Immediatly after the contamination plants were watered.The water droplets must stay on the leaf surface during 12 hours a day. To have a good infection the plants must grow very slowly. So, limited amounts of water must be provided according the weather variations. In Lusignan we sprayed tap water during 2 to 5 minutes periodically during the night (22 h., 23 h., 24 h., 1 h., 2 h., 3 h., 4 h., 5 h.). From 2 to 4 mm of water were supplied every day.

When the symptoms were stabilized to their highest level, each single plant was scored with a scale from 9 to 1 :

9 : no symptom
8 : one leaf with a red needle
7 : less than ¼ of the leaves with red needles
6 : ¼ - ½ of the leaves with red needles
5 : ½ of the leaves with red needles
4 : ½ - ¾ of the leaves with red needles
3 : ¾ of the leaves with red needles
2 : more than ¾ of the leaves with red needles
1 : all the leaves have red needles

2)-The experiment

We wanted to know what dose of inoculum can discriminate the resistance to the red thread disease of varieties of *Lolium perenne* and *Festuca rubra*.

We choosed two turf varieties of these two species. For *L. perenne* 'Limage', more resistant, and 'Elka', more susceptible. For *F. rubra* 'Rubina', more resistant and 'Ensylva', more susceptible (GONDRAN, 1994). We planted the germinated seeds in greenhouse on the 27th September 1993. In order to homogeneize the plant growth we cut

them at 2.5 cm high on the 19th November 1993, on the 03th January 1994 and on the18th February 1994.

The sterilized French bottles of barley grain were seeded with two isolates of *L. fuciformis*. One was collected from a diseased plant of red fescue (n°1) in Lusignan on the 07th of December 1992. The other one (n°2) was isolated on spring 1991 from a diseased plant of *L. perenne* in Les Alleuds (near Angers).

We infected the plants on the15th of March 1994 in a greenhouse with three doses of humid inoculum made with the two isolates : 23 g/dm², 46 g/dm², 92 g/dm². To have an idea of the pathogenicity of the dry inoculum, we scattered the one prepared with the isolate of *L. perenne* at the dose of 3.5 g/dm². Immediatly after the distribution of the inoculum we hourly watered during one minute from 10 p.m. to 6 a.m.. In total 2 mm of water were sprayed every day. On 11th of May 1994, the plants were moved outside. Then every day, water was sprayed 5 minutes per hour during the nights. As the experiment stayed outside from the 11th of May 1994 to the 28th of August 1994, in total, including the rains, plants received 235 mm of water.

The plants were scored for symptoms on the 06th of May 1994, 25th of May 1994, 11th of July 1994, 05th of August 1994 and 28th of August 1994.

The experiment was a split plot with three blocks. Each block was splitted with the inoculum of each isolate.There was a non infected control for each block. Each elementary plot contained two trays of 20 plants which gives a total of 192 trays.

The results were calculated on the averages of scorings and on the percentages of plants without symptom.

RESULTS

1)-Isolate n°1, from a diseased red fescue plant. With the isolate from the red fescue the first symptoms occured on the 06th of May 1994. But at this time the infection was too low and too heterogeneous to reveal significant varietal differences. A similar situation was observed on the 25th of May 1994. On the 05th of August 1995 the varieties ranked as in the field only with the dose of humid inoculum of 92 g/dm² (table 2). At that time for the lower doses of humid inoculum (46 g/dm² and 23 g/dm²) the two varieties of *L. perenne* appeared more resistant than the two of *F. rubra* but no significant differences

could be observed between the two varieties within each species. On 28th of August 1994 the three doses of humid inoculum indicated *L. perenne* more resistant than *F. rubra* but within each species the varieties could not be distinguished.

Table 2 : Infection with humid inoculum of the isolate n°1 from the red fescue (92 g/dm²). Scoring on the 05th of August 1994.

Varieties	Species	Scoring averages	% of plants scored 9 (*)
Limage	*L. perenne*	8.6 a	72 a
Elka	*L. perenne*	7.5 b	18 b
Rubina	*F. rubra*	7.6 b	26 b
Ensylva	*F. rubra*	6.4 c	0 c

a, b, c : Significant differences by t test (P<0.05).

* : Plants without symptom.

2)-Inoculum prepared with the isolate n°2 from a diseased ryegrass plant. The symptoms began to appear on the 06th of May 1994 and remained visible until the 28th of August 1994. But this infection could not distinguish the resistances of the four varieties at the five scoring dates whatever the doses (23 g/dm², 46 g/dm² and 92 g/dm²).

3)-Dry inoculum prepared with the isolate n°2 from a diseased ryegrass plant. With the dry inoculum prepared with the isolate from perennial ryegrass the first symptoms appeared on the 06th of May 1994. But, at this time, the infection was much too weak to separate the varieties. The differences could only be evaluated on the 25th of May 1994 and on the 11th of July1994 (table 3). On the 25th of May 1994 'Limage' was more resistant than 'Elka', but the susceptibilities of the two varieties of *F. rubra* were similar. On the 11th of July of 1994 the process was reversed : 'Limage' was close to 'Elka' and 'Rubina' showed less damages than 'Ensylva'. On the 05th of August 1994 and on the 28th of August 1994 the two varieties of *L. perenne* were less infected than the two varieties of *F. rubra*, but the damages were the same within each species.

Table 3 : Infection with dry inoculum of the isolate n°2 from perennial ryegrass at the dose of 3.5 g/dm². Scoring on the 25th of May 1994 and on the 11th of July 1994.*

Varities	Species	Scoring averages		% of plants scored 9(**)	
		25/May/94	11/July/94	25/May/94	11/July/94
Limage	*L. perenne*	8.7 a	8.6 a	73 a	66 a
Elka	*L. perenne*	7.9 b	7.8 ab	30 b	29 ab
Rubina	*F. rubra*	7.7 bc	7.2 b	27 b	18 ab
Ensylva	*F. rubra*	7.1 c	4.5 c	13 b	1 b

a, b, c : Significant differences by the t test ($P<0.05$).

* : This dose is equivalent at 10.5 g/dm² of humid inoculum.

** : Plants without symptom

DISCUSSIONS AND CONCLUSIONS

1)- Environmental conditions for a good infection

In our experiment we infected the plants in greenhouse on the 15th of March 1994 instead to the end of June in field conditions. During the spring time, in greenhouse the averages of the temperatures were very close to these observed during the summer in field conditions. But the differences between the day and the night temperatures were larger in the field during the summer than in the greenhouse during spring. We observed in the greenhouse the first symptoms 1.5 months after the spreading of the inoculum. But the infection was heterogeneous and weak. Then the plants were transported outside in natural conditions. After two weeks outside the symptoms increased and reached their maximun level for the isolate 2 from perennial ryegrass. For the isolate from red fescue the maximun level of infection was reached later.

These observations meat those of Bahuon (1986) who found that temperatures of 6°C during the night and 18°C during the day were necessary to get a good infection.

2)-Fungus isolates, moisture content, doses of inoculum and time of scoring when the plants have been kept in the open air

The cultivars can be distinguished as in the field with the humid inoculum prepared with the isolate from diseased red fescue plant at the highest dose of 92 g/dm². This result is also possible with the dry inoculum prepared with the isolate from diseased perennial rye grass at the low dose of 3.5g/dm². This dose is equivalent to a humid inoculum dose of 10.5 g/dm². Moreover the time of scorings from the date when the plants were in the open air needs about three months for the humid inoculum prepared with the isolate n°1. About ½ month were necessary for the dry inoculum prepared with the isolate n°2 to distinguish the varieties of the ryegrass.

Before concluding we must repeat an experiment using dry and humid inoculum prepared with these two isolates at different doses. Besides we must infect a larger number of varieties.

3)-Specific resistance of red fescue and perennial ryegrass

Both isolates n°1 from red fescue and n°2 from perennial ryegrass gave the same ranking of the cultivars of red fescue and of the perennial ryegrass. These two isolates are not representative to say any thing about the specific resistances of these turf grass species.

4)-Development of the test

Natural infections are very heterogeneous and the varietal resistance are assesed with many difficulties.

When the test will give constant results it will be very useful and very convenient to breed new varieties for resistance to red thread disease.

Moreover presently we know nothing about the genetics of this disease resistance. An efficient and convenient test will be very useful to study it.

REFERENCES

BAHUON A., 1986. Etude des conditions de propagation de *Corticium fuciforme* (Berk.) Wakef.. Mise au point d'une méthode d'infection artificielle de *Lolium perenne* L. et *Festuca rubra* sp.. Thèse de Docteur de l'Université de Poitiers F. (PhD Thesis, University of Poitiers). 121 p.

GONDRAN J., 1994. La maladie du fil rouge des graminées utilisées comme gazon. Résultats 1993, projets 1994. Publications à tirage limité du laboratoire de Pathologie de Lusignan, 20 p.

GONDRAN J. et COURTILLOT M., 1994. Important diseases of turfgrasses in France. Conf. on harmful and beneficial micro-organisms in pastures, turf and grassland, Paderborn (All.) 4-5 Oct. 1993, IOBC/WPRS Bulletin, INRA, Montfavet (Fr.), 17(1), 71-74.

GONDRAN J., 1995. Les maladies du fil rouge et des taches roses des graminées à gazon. Phytoma, 473, 37-39.

O'NEILL N.R. and MURRAY J.J., 1985. Etiology of red thread and pink patch diseases in the United States. Proc. of the fifth international turfgrass research conference. Avignon Fr. éd. INRA, 595-607.

SMITH J.D., JACKSON N., WOOLHOUSE A.R., 1989. Fungal diseases of amenity turf grasses. Ed. E. and F.N. SPON, London, New York, 401 p.

ACKNOWLEDGEMENTS

We are indebted to Prof. G. RAYNAL (INA-INRA-Paris-Grignon) and to Dr. C. HUYGUE (INRA-Lusignan) for their kind help and constructive comments.

Development of a method for early selection of *Dactylis* breeding material with resistance against *Mastigosporium* spp.

U. Kastirr, F. Ehrig, J. Schubert, U. Schütze

Mastigosporium spp. are widespread fungi on cocksfoot (*Dactylis* spp.) and other grasses. They cause leaf flecks and yellowing of leaves.

In 1963 Schneider and Meyer described a striking disease on cocksfoot in Germany. Two years later, Buhl and Lange reported on eyespots on cocksfoot leaves in monocultures. That infection resulted in high yield losses.

In 1988 Huss et al. detected *Masticosporium muticum* in meadows in Austria, where the yield reduction reached 40%.

Our research on occurrence and impact of *Mastigosporium* species on cocksfoot was the basis for the development of selection methods.

In table 1 fifteen isolates of *Mastigosporium* species are shown which we have found on different grass host plants. These can be subclassified in 4 *Mastigosporium* species.

Table 1: Proof of *Mastigosporium* - Isolates

***Mastigosporium* - species**	**host plants**	**isolates**	**origins**
M. album Riess	Alopecurus pratensis L.	1	Tschechien
M. muticum (Sacc.) Gunnerb.	Dactylis glomerata L.	1	Schleswig-Holstein
		1	Niedersachsen
		2	Mecklenburg-Vorpommmern
		2	Brandenburg
		3	Sachsen-Anhalt
		1	Sachsen
		1	Baden-Württemberg
M. kitzebergense Schlöss.	Phleum pratensis L. Dactylis glomerata L.	1	Baden-Württemberg
M. rubricosum (Dean.etBarth) Nannf.	Agrostis spp.	2	Sachsen-Anhalt

To differentiate these species we have characterized the isolates acording to morphological, biological, biochemical, serological and molecular biological properties.

The several species of *Mastigosporium* can be differentiated quite clearly in terms of **morphology**.

We have found differences between types of mycelium growth, size and development of conidia and have identified isolates of the species of *M. album, M. kitzebergense, M. muticum* and *M. rubricosum*.

In figure 1 are shown the different symptoms, which cause the fungi species on several host plants, the different types of mycelium and conidia.

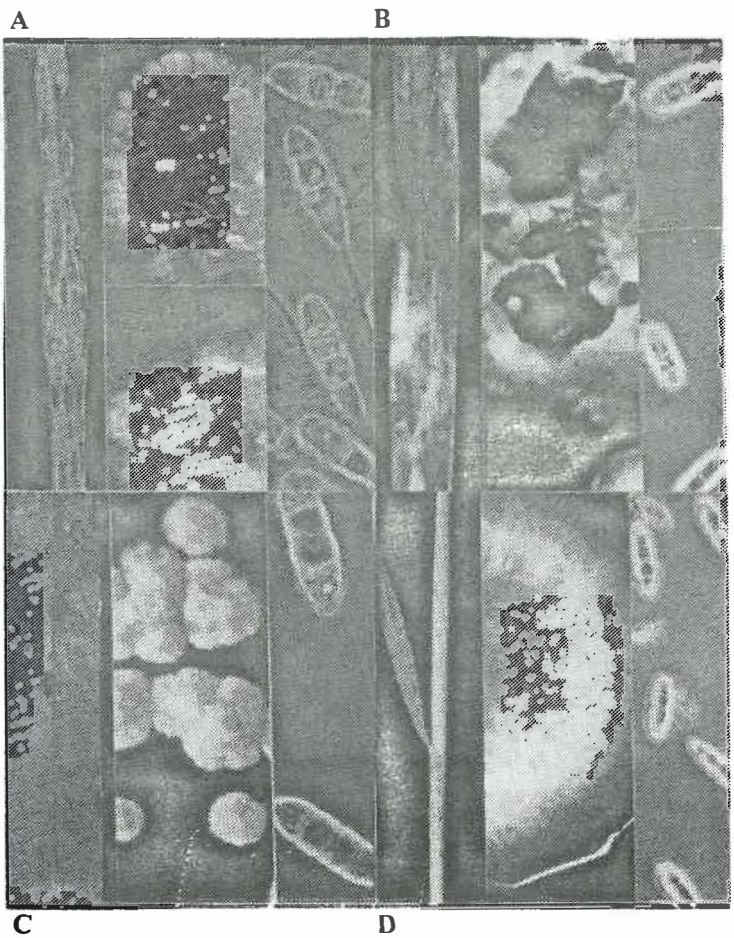


Figure 1: Comparison of different types of mycelium and conidia of *Mastigosporium* species
A - *M. album*
B - *M. kitzebergense*
C - *M. muticum*
D - *M. rubricosum*

The conidiophores of *M. album* are short, solitary or in group. The apical cell or sub-apical cell of conidia bears a single or bifurcated hyalin appendage. The conidia form slimy masses.

The short conidiophores of *M. kitzebergense* are born on the main hyphae or side hyphae of mycelium and they constict typical Alourioconidia.

The short conidiophores of *M. muticum* are born on narrow penetration hyphae.They constict one daugther cell after the other from the same mother cell. We have found conidiophores with swellings in shape of 3 to 7 anneliding rings after the building of conidia.

For the **early selection of breeding material** for resistance against *M. muticum* the following method of inoculum production and inoculation of detached leaves or seedlings was developed. The cultivation and multiplication of fungal isolates were used in malt pepton liquid. For the production of the inoculum the mycelium from liquid culture was transmitted in malt pepton agar at 20°C and black light (320 - 400 nm).

After 3 weeks conidia were rinsed with tap water for a conidia suspension with 10^6 cells/ml. Plants were infected with spray inoculation and for several days they were covered with foil to ensure a high humidity of the air. The detached leaves were inoculated in a damping box.

After 4 days the symptoms started to develop. The fungal hyphae penetrate the leaf surface and are building up conidiophores and conidia. After 7 days developed white centres in the brown leaf spots with enormous numbers of conidia (fig.2).

With this infection method 48 species and more than 200 clones of cocksfoot were selected and assessed with a scoring scale. We have researched the variability of disease severity. With the help of a progress curve for a defined area infected by disease we can safely maintain that there are clear differences between tolerant and susceptible genotypes against *M. muticum* (fig.3).

These differences show that the selection for resistance against *M. muticum* on cocksfoot is successful.

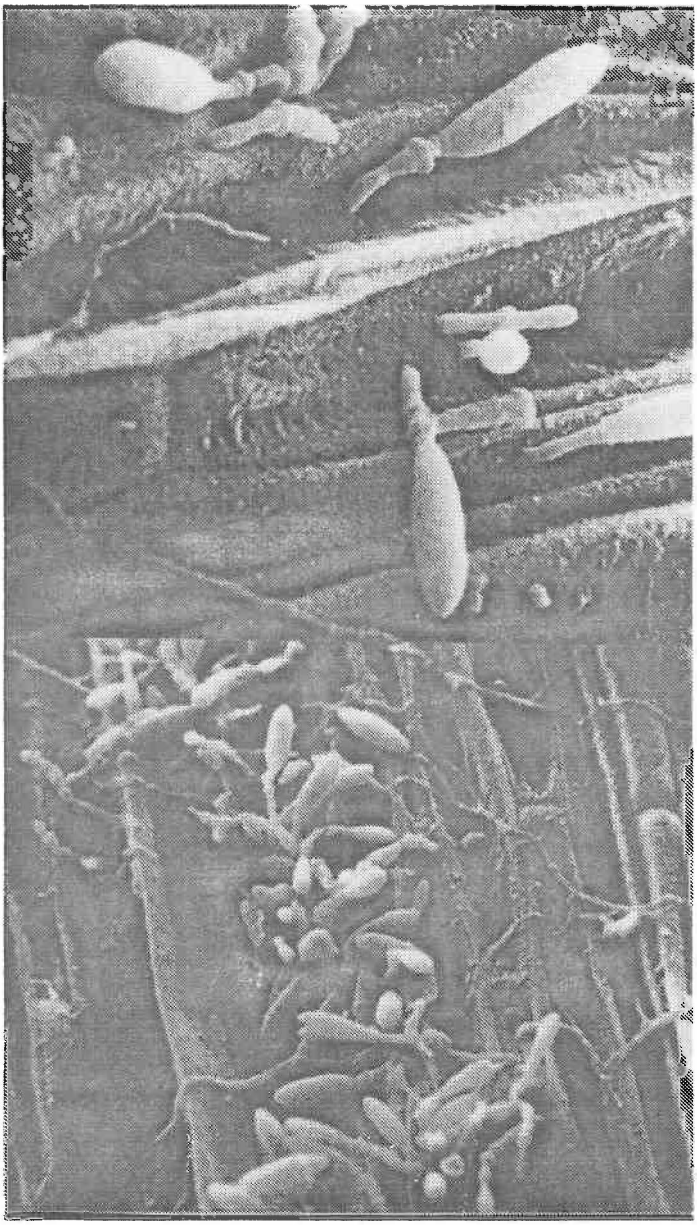


Figure 2: Development of *M. muticum* on leaves after spray inokulation with conidia suspension
A - 4 days after inokulation; B - 7 days after inokulation

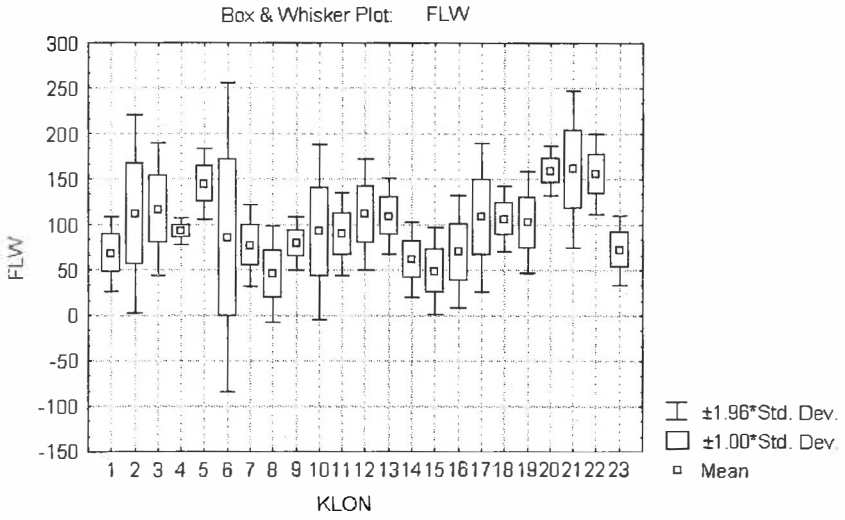


Figure 3: Variability of disease severity different cocksfoot clones
high susceptible - 5, 19 - 22; less susceptible - 1, 8, 14 - 16, 23

In the **biological test** for susceptibility of different host plants against several *Mastigosporium* species we found that the species specialize on the respective host plant. In table 2 are collected the host plants of *Mastigosporium* species.

Table 2: Host plants of *Mastigosporium* - species

host plants	***Mastigosporium* species**			
	M. album	**M. muticum**	**M. kitzebergense**	**M. rubricosum**
Agrostis alba L.	-	-	-	+
A. gigantea Roth.	-	-	-	+
A. stolonifera L.	-	-	-	+
A. tenuis Sibth.	-	-	-	+
Alopecurus pratensis L.	+	-	-	-
Arrhenatherum elatius (L.) J. et C. Presl	+	-	-	-
Dactylis glomerata L.	-	+	+	-
D. aschersoniana L.	-	+	-	-
Lolium perenne L.	-	-	-	-
Phleum pratanse L.	-	-	+	-

M. album can infect only *Alopecurus pratense* and *Arrhenather*um elatius. *M. muticum* only *Dactylis* spp., *M. kitzebergense Phleum pratense* and *Dactylis glomerata* and *M.rubricosum* several *Agrostis* species.

For the **serological detection** of *Mastigosporium* species in cocksfoot leaves a polyclonal antiserum was raised in rabbits. As antigen for the immunisation a homogenate of fungal mycelium in physiological sodiumchlorid solution was administered four times intramuscularly. Antisera were collected 10 days after the last immunisation and IgG were prepared according to standard methods. All together four rabbits were immunised. The antisera showing the highest titer were used for the development of an indirect ELISA (plate trapped antigen) PTA-ELISA without precoating with IgG. The cross-reactivity with 17 fungal species was tested in PTA-ELISA and the results are presented in table 3.

Table 3: Cross - reactivity screening of Mastigosporium - antiserum

fungal pathogen	ELISA - reactivity	Extinction by E450
Alternaria sp.	(+)	0,15
Cladosporium phleii	-	0,03
Corticium fuciforme	-	0,05
Drechslera teres	-	0,01
D. graminearum	-	0,01
Erysiphe graminis	-	0,03
Fusarium sp.	-	0,02
Mastigosporium album	++	0,80
M. muticum	+++	1,80
M. kitzebergense	+++	1,70
M. rubricosum	+++	1,80
Monililia sp.	(+)	0,18
Pseudocercosporella sp.	+	0,23
Puccinia striiformes	-	0,05
P. perplexans	-	0,04
Penicillium sp.	+	0,40
Rhynchosporium sp.	++	0,90
Trichoderma viridae	-	0,05
Verticillium sp.	+	0,30
Pufferkontrolle	-	0,05

The assay showed a good reactivity with the 4 *Mastigosporium* species in infected leaves or as a mycelium culture. With the exception of special *Rhynchosporium secalis* and 5 others fungal species the polyclonal IgG preparation failed to react with 7 fungi. Because of this serological cross-reactivity the assay does not seem to be not suitable for detection of *Mastigosporium* in field samples, which are co-infected with *Rhynchosporium* spp.

The **biochemical properties** were tested concerning the formation of extracellular enzymes in planta. In figure 4 make clear, that during the process of infection the fungi produce Xylanase.

Between the isolates differences in the extracellular enzymatic activity are clearly recognizable. But the correlation to the pathogenicity of isolates in planta is not significantly proved.

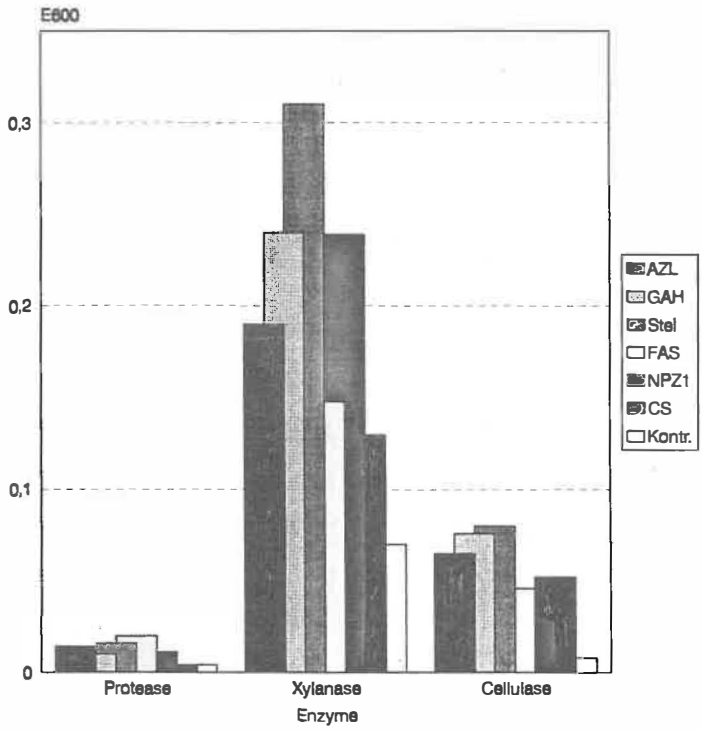


Figure 4: Enzymatic activity several *Mastigosporium* isolates in planta 4 weeks after inoculation

The aim of our **molecular biological research** was to work out a PCR approach for differentiation of species and isolates of *Mastigosporium* differing in origin, pathogenicity and host specificity.

Using purified fungal DNA different random primers (n= 10-16) were tested in PCR. Among them (GACA)4 was the best suited to differentiate between both investigated *Mastigosporium* species, *M. muticum* and *M. kitzebergense*. Application of this primer resulted in PCR for both species in bands of identical and different sizes. Two of the bands allowing to differentiate between both species, one from *M. muticum* (300 bp) and one from M. kitzebergense (1600 bp), were isolated and cloned in *E. coli* and sequenced. On the basis of the sequence data two pairs of primers were synthesized. These primers enabled us to differentiate both species if purified DNA from fungal cultures was used in PCR.

In a further experiment we tested the usefulness of the primers in detection of the fungi in plant. For this purpose we used the primer pair specific for *M. muticum* and purified DNA from healthy and infected plants. The result of the PCR is shown in fig.5. It shows that it is possible to detect the fungus in plant material and to identify the corresponding fungal species. After simplification of the purification method for plant DNA it should be possible to use this method in routine testing.

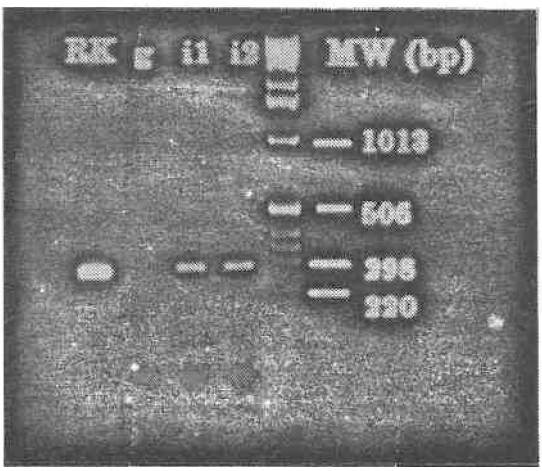


Figure 5: Detection of *M. muticum* in infected plants by PCR
RK - DNA from mycelium; g - DNA from healthy plants;
i1/i2 - DNA from infected plants

Leaf Spot Resistance in Grasses

U.K. Posselt

University of Hohenheim (720), 70593 Stuttgart, Germany

Abstract

Leaf spot caused by *Drechslera spp.* was investigated in italian and perennial ryegrass and in meadow fescue. Neither in inbred nor in noninbred material fully resistant plants could be detected. Progenies from diallel matings were tested for resistance to *D. siccans*. In most of these experiments significant genetic variation could be found. By further partioning into gca- and sca-variance it became obvious, that in most cases sca was more important than gca. Additionally, ambidirectional dominance could be demonstrated to be present in particular materials. Heritability estimates were in the order of 0.3 to 0.5. In one of the selfed progenies bimodal segregation for leaf spot resistance occured. The implications of these results in respect to breeding strategies are discussed.

Keywords: Drechslera spp., italian and perennial ryegrass, meadow fescue, genetic variance, gca, sca, heritability

Introduction

Leaf spot diseases are very common under temperate climatical conditions, especially during cool and wet periods (LEWIS, 1992). The diseases negatively affect both yield and quality (COOK, 1975; LAM,1985) Leaf spot deseases are caused by various pathogenic fungi among which *Drechslera spp.* are the most important ones. Artificial inoculation experiments were carried out to study i) the amount of genetic variation, ii) the importance of gca vs. sca-variance and iii) the possibility of improving disease resistance in different grass species.

Materials & Methods

Plant material

Host plant materials were inbred lines of italian and perennial ryegrass (*Lolium multiflorum & L. perenne*) and non inbred plants from cultivars of meadow fescue *(Festuca pratensis)*. Diallel matings and selfing of parental plants were carried out in the glasshouse by mutual pollination under paper bags. Preselected families were multiplied in seed islands in a field of rye.

Inoculum production

Diseased plant material was collected in the breeding nurseries. Besides *Drechslera andersenii* most isolates could be identified as *D. siccans*. Maintenance of the various isolates took place in test tubes on soil or by means of cryoconservation storage in liquid nitrogen at -196 °C. Isolates were tested for aggressiveness before multiplication. Inoculum production took place in a two step procedure: First, production of mycelium and spores on PDA-medium under UV-light. Second, transfer of mycelium plugs into Erlenmeyer flasks with liquid vegetable juice. The cultures were kept on a rotary shaker (80 - 100 rpm) for about one week and again under UV-light. The mycelium was homogenized with a Waring blender. Several isolate suspensions were mixed and used as a bulk inoculum. Inoculation was performed by spraying the mycelium-spore-suspension by means of a paint sprayer pistol on 10 - 12 week old plants (2 ml per plant).

Inoculation experiments

They were carried out in a glasshouse compartment with partial control of light and temperature. After inoculation the plants were kept under a plastic tunnel to maintain high humidity for 2 days. First lesions were visible after 3 days and scoring was done after two weeks. The amount of lesions were estimated as % diseased leaf area (DLA) according to the following scale:

score	1	2	3	4	5	6	7	8	9
% LAI	0-1	1.1-3	3.1-6	6.1-10	10.1-15	15.1-20	20.1-30	30.1-50	>50.1

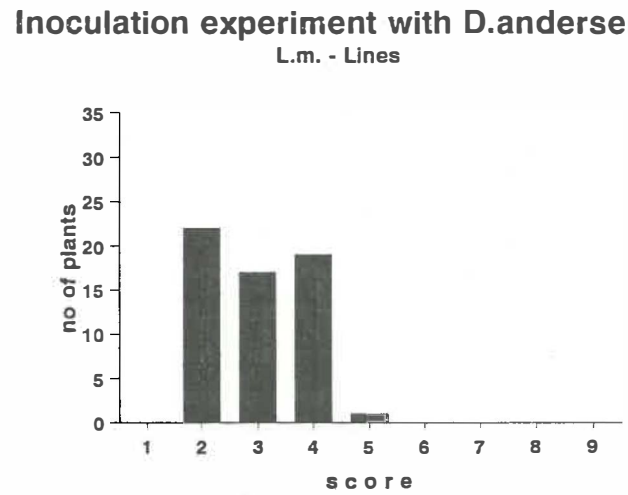
Statistics

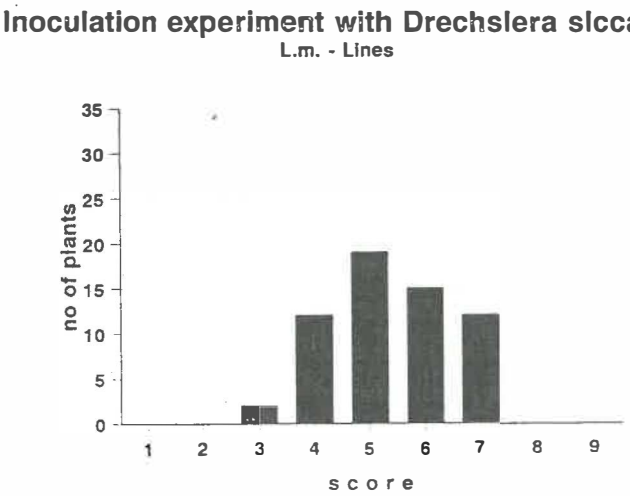
All computations were done with PLABSTAT (UTZ, 1990). Diallels were calculated according to Griffing's method 4, model 1(GRIFFING, 1956).

Results

Comparison between Drechslera spp.

A set of 30 inbred lines of italian ryegrass was inoculated with both *D. andersenii* (D.a.) and *D. siccans* (D.s.). As can be seen from fig. 1, infection with D.s. was much more severe than that of D.a. (5.6 vs 3.2). Because of the broader range of resistance among the lines it was decided to use only *D. siccans* in all other experiments.





Crossing experiments

A set of 5 inbred lines of italian ryegrass was intercrossed in a diallel manner and the F1 progenies together with the parental lines were inoculated. The results are given in table 1.

Table 1 : Leaf spot resistance of inbred lines of L. multiflorum and their F 1's (% DLA)

	1	2	3	4	5	GCA
1	**20**	14	18	16	16	-0.96
2		**19**	21	19	17	1,29
3			--	18	17	2,09
4				**19**	12	-0,81
5					**17**	-1,62
M	16	18	18	16	15	17

LSD 5 = 2.8

ANOVA

	MS	VC
CROSSES	22.09	4.53**
GCA		1.27^{ns}
SCA		2.84**
ERROR	3.95	

Since breeders are mainly dealing with heterozygous materials, crossing experiments were carried out with unselected plants from two meadow fescue cultivars according to a diallel mating design.

In table 2, the results of the inoculation experiments are given in a diallel table including the 6 parental plants from cv. Predix on the diagonal. Parents nos. 3 and 2 are those with the best gca-values, i.e. highest gca for *D. siccans* resistance. Among the genetic variance sca was much more important than gca. If one looks to individual crosses it is quite obvious that some parents show ambidirectional dominance i. e. P 6 x P 5 results in a progeny with much higher resistance than both parents, while the progeny of P 6 x P 1 is more susceptible than both parents.

Table 2: Leaf spot resistance of the diallel progeny of 5 noninbred plants of cv. Capella (% DLA)

	1	2	3	4	5	GCA
1		14	15	17	15	0.4
2			15	20	10	0.2
3				16	10	-1.2
4					17	3.5
5						-2.6
M	15	15	14	18	13	15

LSD 5 = 2.8

ANOVA

	VC
CROSSES	6.53**
GCA	3.68^{+}
SCA	4.09**
ERROR	1.62

The results of the 5 x 5 diallel of plants from cv. Capella are slightly different. In this case, unfortunately the parents couldn't be maintained and tested together with their F1's. Genetic variation and sca-variance (see table 3) is of the same magnitude as for the Predix-diallel. Though sca-variance is more important than gca, the latter too is of significance. Studying the diallel table as given in table 3 one can easily imagine that in this material ambidirectional dominance is present, too.

Selfed progenies

In table 4 the segregation pattern of the selfed progenies of 6 heterozygous plants are given. While in the progenies 2 to 6 a single peak occurs, the situation in progeny 1 is rather different. Here, a bimodal distribution could be detected, explaining the importance of sca (i.e. dominance) which was found in the respective crossing experiments.

Table 3: Leaf spot resistance of 6 noninbred plants of cv. Predix and their diallel progeny (% DLA)

P	1	2	3	4	5	6	GCA
1	**16**	15	11	19	20	20	2.3
2		**21**	13	17	13	12	-1.2
3			**15**	12	15	13	-2.8
4				**17**	16	16	1.2
5					**18**	13	0.6
6						**18**	-0.2
M	17	14	13	16	15	15	15

LSD 5 = 4

ANOVA

	MS	VC
CROSSES	35.15	6.85**
GCA		1.75^{ns}
SCA		4.34*
ERROR	7.7	

Heritability

From various crossing experiments within perennial ryegrass and meadow fescue heritabilities were calculated. They ranged from 0.27 to 0.53. Having in mind that these figures originate from replicated trials, then it is quite clear that heritability on a single plant basis is rather low for resistance against *D. siccans*.

Population improvement

Single plants from cultivars Capelle, Predix and Poseidon (meadow fescue) as well as from Kerem and Marietta (perennial ryegrass) were screened for resistance. The less susceptible fraction, consisting of 10 to 39 plants per population were selected, intercrossed and their respective progenies tested. In none of the five populations (see table 4) significant improvement could be made.

Table 4: Leaf spot resistance of selfed progenies of cv. Predix
(% DLA)

%	1	2	3	4	5	6
0 - 1						
1 - 3						
3 - 6						
6 - 10	3				1	
10 - 15	8	3	10	5	4	11
15 - 20	4	17	6	14	27	23
20 - 30	7	22	4	5	18	13
30 - 50	2	7				3
> 50	1					
mean	16	21	15	17	18	18
no. of plants	24	50	20	24	50	50

Table 5: Population improvement in cultivars of meadow fescue and perennial ryegrass

	No. of families	% DLA progenies	% DLA cultivar	difference
Capella	39	10.7	11.7	n.s.
Predix	16	7.9	6.6	n.s.
Poseidon	16	9.6	7.4	n.s.
Kerem	10	13.5	15.7	n.s.
Marietta	10	12.2	10.8	n.s.

Discussion

Resistance to *Drechslera siccans* in several Lolium and Fescue species has been descibed by WILKINS (1973). However, almost no information about the amount of genetic variation and the importance of gca and sca is available. In tall fescue, tested for resistance to *D. sorokiniana*, LINSCOMBE et al (1982) found considerable genetic variation among diallel progenies. In their study gca was predominant for lesion coverage and lesion size which let them conclude that mass selection is an efficient means for improving the level of resistance. This is contrary to the results from the infection

experiments in italian ryegrass and meadow fescue where the resistance to D. siccans was investigated. In most experiments the largest part of the genetic variation was due to sca. General combining ability was mostly non significant and always smaller than specific combining ability. The implication of these findings are not very encouraging for plant breeders since it means that prediction of the level of resistance is not feasable. From the diallel tables it is quite obvious that ambidirectional dominance occurs. This means that the direction towards either resistance or susceptibility in the progeny is unpredictable. The segregation pattern of selfed progenies implies polygenic inheritance. However, in one offspring a bimodal distribution confirms the presence of major genes with dominant effects. The presense of ambidirectional dominance together with low single plant heritability for leaf spot resistance are the possible explanations for the poor response to population improvement by simple mass selection. In an selection experiment with S1-families tested in replications (data not given) a significant improvement in the level of resistance could be demonstrated. Breeders are therefore adviced to apply full- or halfsib family selection.

References

Cook, F.G. 1975: Production loss estimation in Drechslera infection of ryegrass. Ann. appl. Biol. 81: 251-256.

Griffing, B. 1956: Concept of general and specific combining ability in relation to diallel crossing systems. Austral. J. Biol. Sci. 9: 463-493.

Lam, A. 1985: Effect of fungal pathogens on digestibility and chemical composition of Italian ryegrass (*Lolium multiflorum*) and tall fescue (*Festuca arundinacea*). Plant Pathology 34: 190-199.

Lewis, G.C. 1992: Foliar fungal diseases of perennial ryegrass at 16 sites in England and Wales. Crop Protection 11: 35-38.

Linscombe, S.D., C.E. Watson,Jr., and L.E. Trevathan 1982: Inheritance of resistance to infection by *Drechslera sorokiniana* in tall fescue. Crop Sci.23: 1085-1087.

Utz, H.F. 1990: PLABSTAT. Version 2B. Inst. f. Pflanzenzüchtung. Universität Hohenheim.

Wilkins, P.W. 1973: Infection of *Lolium* and *Festuca* spp. by *Drechslera siccans* and *D. catenaria*. Euphytica 22: 106-113.

BREEDING PERENNIAL RYEGRASS WITH A BETTER CROWN RUST RESISTANCE

Dirk Reheul and An Ghesquiere

Ministry of Agriculture - C.L.O.-Gent
Rijksstation voor Plantenveredeling
Burg. Van Gansberghelaan 109
B-9820 Merelbeke

SUMMARY

Improving the crown rust resistance is one of the main goals in our breeding programme. We select both after artificial infections and after natural epidemics on several locations. Hence we present the relationship between :
- the rust resistance in parents and their offspring
- the rust resistance and the dry matter yield
- the rust resistance and the digestibility.
Furthermore we present data on the variability of the rust resistance during several years and on several locations and on the relevance of the artificial infections.
All results are based on large numbers of observations.

INTRODUCTION

Crown rust (*Puccinia coronata Corda*) is a widespread foliar disease on ryegrasses in Western-Europe. Breeding for a better rust resistance is a goal in most breeding programmes with ryegrasses.
The disease used to attack the ryegrass during September and October in Belgium. Recently we have had epidemics as early as in the beginning of July. These early infections were sometimes followed by a second epidemic starting from the end of August on.

Having 2 cycles of infection per year offers the opportunity to select sharply, provided that the genotypes react similarly during both epidemics. We found more than once important discrepancies between the infection rates during 2 consecutive epidemics. The same holds true for rust epidemics during consecutive years. And not surprisingly, differences between locations are well known. Moreover, the disease spreads irregularly over the field and if one does not use spreader plants, plots or rows the assessment of the infection is unreliable.
For all these reasons, the selection of the right genotypes is not as easy as it looks like.
Artificial infection (AI) can speed up the selection by adding an extra infection beyond the growing season. It is a prerequisite that the results of an artificial (AI) and a natural infection (NI) are corresponding.
A mild negative selection after AI followed by a positive selection after a NI improves the efficiency of a selection programme.

According to WILKINS (1975) the inheritance of crown rust resistance is mainly based on a relatively large number of minor genes. HAYWARD (1977) confirmed the quantitative character of the reaction on the disease as did SCHMIDT (1980), CHOSSON and SINGLA (1989) and POSSELT (1994) citing similar results obtained by MANSAT and BETIN (1979). However dominant major genes may be present (WILKINS, 1975 ; HAYWARD, 1977 ; POSSELT, 1994). HAYWARD (1977) cites a narrow sense heritability of 0.58 offering a high potential for improving the rust resistance.

Creating synthetic varieties is making compromises, weighing the value of different components and the rust resistance is but one of the characteristics involved. We were very interested in the potential scope to create healthier varieties without paying too high a price on the yield and the quality level.
We calculated the realized narrow sense heritability of the rust resistance starting from a set of clones with a presumed high breeding value. Furthermore we quantified the expected genetic consequences both on rust resistance, and yield and quality potential by several selection levels.
Large numbers of relations were involved in all measurements improving the reliability of the results.

MATERIAL AND METHODS

A part of our breeding programme with forage grasses is run as a polycross-progeny testing system. We select plants from a spaced plant nursery and propagate them into replicated clonal rows. The clonal rows (consisting of 5-7 individuals) are planted in lanes. We sow a line of a very rust susceptible variety perpendicular to these lanes. As a consequence each clonal row is guarded by the rust source at both ends. Selected clones are polycrossed and the seed is harvested separately. The offspring is observed in the following seasons in yield trials. Apart from the yield, other important characteristics as digestibility are studied. The digestibility is analysed in all cuts with the Near Infrared Spectroscopy (NIRS) method based on a pepsine cellulase calibration set.
In the mean time the clones are conserved to construct varieties later on.
From the 1992 seed harvest on, all progenies were artificially infected with crown rust spores during the winter. Our AI is a modified procedure of the method of BIRKENSTAEDT, 1990. The detailed procedure is described in REHEUL and GHESQUIERE 1994a and 1994b. We used spores grown by the Labor für Biotechnologie und Phytomedizin, Soest, Germany in 1992, 1993 and 1994. In 1995 spores collected in our own fields during the summer of 1994 were dispersed. The spores obtained from Soest represented a pan-European mixture of crown rust pathovars. The infection of the omnipresent standardvarieties allowed us to assess the success of the AI. So 9 series of AI (5,000 plants inoculated per series) with a very comparable degree of infection are discussed in this paper. These series were conducted in 1993, 1994 and 1995.
On average about 40 % of the infected plants were selected. The most resistant plants were selected and planted in a new spaced plants nursery during the next spring.
During the same period the unselected progenies went (a) in yield trials in Merelbeke and (b) in specific rust trials in Merelbeke, Selommes (France) and the Flevopolder (The Netherlands). There were 2 and mostly 3 replicates in all trials.
In the yield trials the genotypes were present as plots of 9 m^2, in the rust trials as rows of 2-3 m length.
As was the case within the clonal field, spreader lanes, paths or rows were abundantly present everywhere in the yield and rust trials to ensure a regular introgression of the rust.

This working method, illustrated in Figure 1, enabled us to study the relationship between:

1. parents and offspring (the offspring regressed on one parent)
2. the rust incidence in the three locations
3. the rust incidence after the AI and the NI
4. the rust incidence between consecutive years in Merelbeke on the same material
5. the rust incidence and the yield and the quality performance.

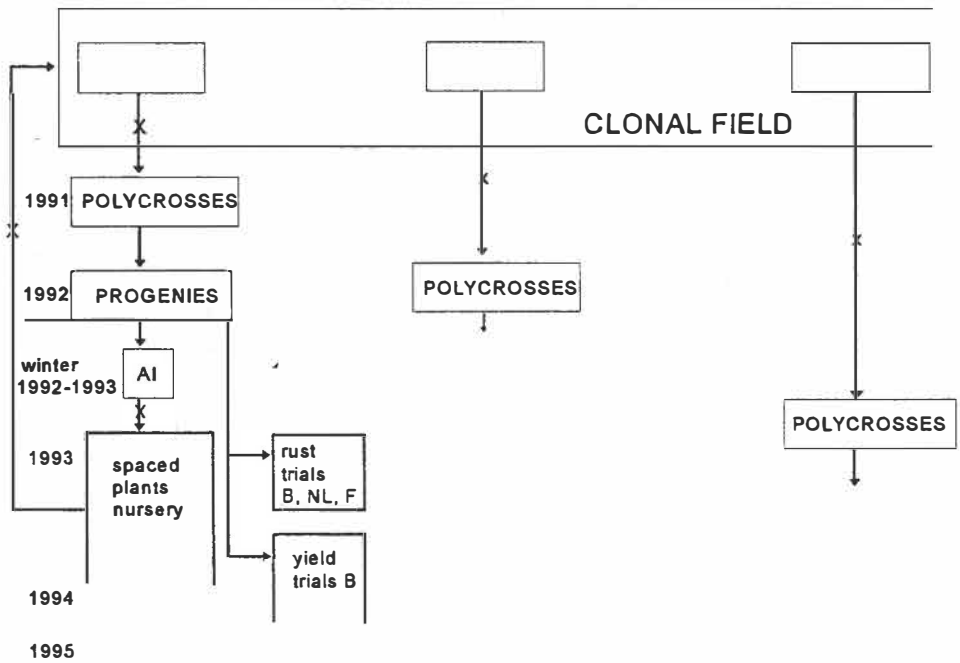


Figure 1 : How the selection and breeding for a better crown rust resistance fits in our breeding programme.
AI : Artificial infection with rust spores ; X : selection

We used a 1 to 5 scale to assess the rust incidence in Merelbeke, 5 being no rust. This system scores the rust resistance.
The rust incidence was scored in a scale of 1 to 9 (1 being no rust) in the Netherlands and in France. Since this scale was mainly used in steps of 2 units (x = 1, 3, 5, 7, 9) it could be converted into the 5 unit scale by the formula (11-x)/2.
Further on we will speak about **rust resistance.** The results presented in this paper are obtained on the basis of the R.v.P. genepool.

RESULTS

1. The variability of the rust incidence

1.1. The variability between years

Table 1 gives the distribution of 1267 clones over the 5 rust classes both in 1994 and 1995. The coefficient of correlation for the rust resistance over the 2 years was 0.53***.

Table 1 : Distribution of 1267 clones over the 5 rust classes (1-5 ; 5 is no rust) both in 1994 and 1995.
rust score in 1994 : 6 September ; mean score 3.02 1.24
rust score in 1995 : 12 October ; mean score 2.80 1.17

	Number of clones				
Rust resistance 1995	Rust resistance 1994				
	1	2	3	4	5
1	67	56	22	12	5
2	61	163	113	79	15
3	20	59	93	79	28
4	7	22	80	112	66
5	0	4	23	38	43

% clones with equal score		37.7
difference	1 unit	43.6
	2 units	15.3
	3 units	3.0
	4 units	0.4

We found a difference in rust resistance of at least 2 units in about 20 % of the clones which we consider as quite much.

We consider genotypes with a rust resistance of less than 4 as inferior.

From Table 1 one can calculate the risk of eliminating potential good clones and the risk of selecting the wrong clones after one year of rust assessment. Indeed, out of a group of 790 clones with a score 1, 2 or 3 in 1994, 136 clones turned into a 4 or 5 in 1995. So the risk of eliminating potential good clones in 1994 was 17 %.

At the contrary 218 clones out of 477 scored as a 4 or 5 in 1994 turned into a 3 or less in 1995. This represents a 46 % risk of selecting inferior clones after 1 year.

This fairly high risk decreases by selecting only the top : 48 clones out of 157 scored as a 5 in 1994 turned into the inferior classes in 1995 which still represents 31 %.

We had no more than 259 clones (20 %) scoring well (4 or 5) in both years and no more than 43 (3 %) maintaining a superior level during both years.

Whatever the reasons for this discrepancy between years may be (different rust pathovars, major genes or both ...) a breeder has to make decisions. The risks of eliminating the right genotypes are not too high if one practices a negative selection.

By selecting the upper classes one takes along 50 % inferior material. The sharper one selects the smaller the risk to select the wrong genotypes. But at that moment one may run into problems by narrowing the genetic diversity with too great a level.

Table 2 : Distribution of 496 families and varieties over the 5 rust classes (1-5 ; 5 is no rust) both in 1993 and 1994
rust score in 1993 : mean 3.41 0.97
rust score in 1994 : mean 3.20 0.85

Rust resistance 1994	Number of families/varieties Rust resistance 1993				
	1	2	3	4	5
1	0	1	1	0	0
2	6	21	20	18	5
3	1	33	77	98	24
4	0	4	26	70	26
5	0	1	5	25	34

We found completely comparable results in our yield trials where we scored the rust resistance in 1993 and 1994 (coefficient of correlation 0.48***). See Table 2.
Out of the group of 300 families scored as a 4 or 5 in 1993, 145 turned in an inferior class in 1994 which equals 48 %. 89 families got a 5 in 1993 and 29 of them (33 %) behaved inferiorly in 1994.
No more than 34 out of 496 performed outstandingly during both years i.e. 7 %.

1.2. The variability between locations (Belgium, The Netherlands and France) in 1993 and 1994

Table 3 summarizes the relationship between the 3 locations.

Table 3 : Coefficients of correlation (r) between the rust resistance in 3 locations during 2 years for different numbers of families and varieties (n)

			NL	B
1993	F	n	150	139
		r	0.44***	0.59***
	NL	n		139
		r		0.56***
1994	F	n	200	177
		r	0.63***	0.57***
	NL	n		177
		r		0.72***

Owing to the large numbers of families and varieties the positive correlation was highly significant, but the value was quite low.
Again some important discrepancies between the rust resistance occurred as can be seen from Figure 2 and Table 4.

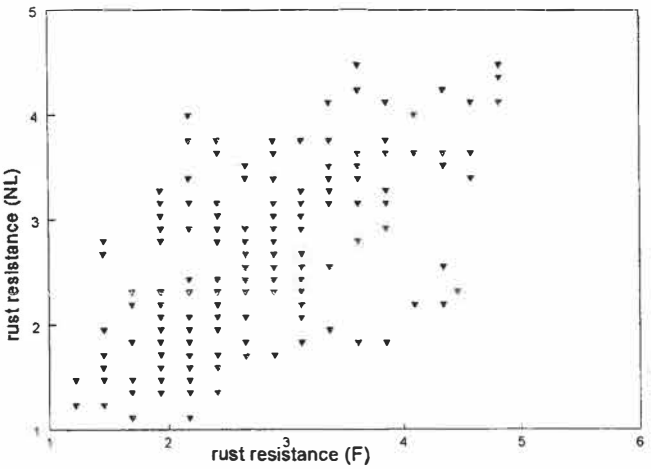


Figure 2 : Relationship between the rust resistance in the Netherlands and in France in 1994

Table 4 : Distribution of 200 families/varieties over the 5 rust classes (1-5 ; 5 is no rust) in France (F) and the Netherlands (NL) during 1994

Rust resistance in NL	Number of families/varieties Rust resistance in F 1	2	3	4	5
1	0	7	2	0	0
2	1	38	29	4	2
3	0	11	56	9	1
4	0	3	11	14	8
5	0	0	0	1	2

% families/varieties with equal score		55.5
difference	1 unit	38.5
	2 units	5.0
	3 units	1.0
	4 units	0

41 genotypes (21 %) were scored with a 4 or 5 in France but 16 of them turned into an inferior class in the Netherlands. Now the opposite. 39 genotypes (20 %) got a 4 or 5 in the Netherlands, 14 of them behaved not well enough in France. On average, about 40 % of the genotypes that were good enough in one location failed in the other.
The relation becomes more complicated if one considers all three locations.

2. The narrow sense heritability of the rust resistance

The parent-offspring regression enabled us to calculate the (realized) narrow sense heritabi-lity : $h^2 = 2b$, FALCONER (1981), b being the slope of the linear regression of the offspring on the female parent. Table 5 summarizes the data.

Table 5 : Parent-offspring relationship for crown rust resistance during several years in several locations

Re-lation	Period of rust assessment m = mean				Lo-cation	Number of pairs involved	b	h^2
	Parents	Rust Trials	Offspring	Yield trials				
(1)	05.09.94	m	18. and 26.08.94	-	B	181	0.24***	0.47
(2)	05.09.94	m	27.08 and 06.09.94	-	F	126	0.32***	0.64
(3)	05.09.94	m	23.08 and 26.09.94	-	NL	126	0.31***	0.62
(4)	05.09.94			17.08.94	B	64	0.18*	0.36
(5)	05.09.94	m	12 and 31.08.93		F	57	0.11 NS	
(6)	05.09.94	m	24.08, 06.09 and 30.09.93		NL	57	0.17**	0.33
(7)	11.10.95		13.10.95		B	117	0.29***	0.59
mean (1), (2), (3), (7)							0.29***	0.58

The mean linear coefficient of regression for relations (1), (2), (3), (7) (parents and offspring compared during the same season in the rust trials)was 0.29***.
Within the initial population of over 300 parental clones an increase in rust resistance of 1 unit was reflected in 0.29 units improvement in the offspring.

The calculated h^2 equals 0.58, confirming the high value found by HAYWARD (1977).
The genetic response (R) can be calculated from the formula $R = h^2S$, S being the selection differential and defined as 0p - 0s ; 0p is the mean value of the parental population ; 0s is the mean value of the selected population.
Table 6 gives the genetic response calculated for relation (1) out of table 5.

Table 6 : Response (R) according to different selection differentials (S) in a population of 181 clones
mean rust resistance (0p) 2.92 ; 1.24 ; $h^2 = 0.47$

Proportion selected (P)			
5	5	2.08	0.98
10	5	2.08	0.98
15	4.70	1.78	0.84
20	4.53	1.61	0.76
25	4.42	1.50	0.71
30	4.35	1.43	0.67
35	4.30	1.38	0.65
40	4.17	1.25	0.59
45	4.04	1.12	0.52
50	3.93	1.01	0.47

Polycrossing the 10 % best clones out of this group of 181 should generate an offspring with a rust resistance improved by 1 unit.
To achieve an advance of 0.75 units one has to polycross the top 20 % of this group.
Rejecting 50 % of the clones results in a potential progress of 0.47 units rust resistance.

3. The relationship between the DMY and the rust resistance

In 9 out of 10 trials we found a negative coefficient of correlation between the annual yield and the rust resistance (Tables 7 and 8).

Table 7 : Information on the yield trials used to calculate the relationship DMY-rust resistance in 1994

Series	Sowing year	Trials	Number o cuts in 1994	Number of families/ varieties	Types E = earlies I = interm. L = lates	Rust resistance date value	Date of the rust cut	DMY (k /ha DM) rust cut	annual	LSD (5%) annual DMY
1	1992	1	4	22	E	19.09.94	19.09	2352	13017	7.2
		2		48	I		19.09	2488	12899	7.2
		2		48	I + L		19.09	2240	12745	8.8
		4		72	L		21.09	2614	12324	6.0
		5		48	L		22.09	2502	12564	7.0
	TOTAL			238				2439 (19 %)	12710	
	MEAN					2.98				
	σ					0.85				
2	1993	6	5	68	E	16.08.94	23.08	3278	18608	7.4
		7		32	I		23.08	3400	18641	5.3
		8		80	I		22.08	3559	18468	7.0
		9		88	L		22.08	3615	18437	6.9
		10		96	L		22.08	2873	17511	6.9
	TOTAL			364				3345 (18 %)	18393	
	MEAN					3.26				
	σ					0.87				

Table 8 : Coefficients of correlation (r) and regression (b) between the DMY (kg/ha) (A, B, C) and the rust resistance in 10 trials
(A) : infected cut ; (B) : all cuts ; (C) : (B) - (A)

Trials	r			b	
	(A)	(B)	(C)	(B)	(C)
1	0.01 NS	-0.23 NS	-0.27 NS	-123 NS	-124 NS
2	0.05 NS	-0.18 NS	-0.20 NS	-156 NS	-163 NS
3	0.10 NS	-0.18 NS	-0.23 NS	-197 NS	-223 NS
4	0.17 NS	-0.23*	-0.30**	-200*	-234**
5	-0.28 NS	-0.29*	-0.22 NS	-222*	-168 NS
MEAN	0.01 NS	-0.22***	-0.24***	-180***	-182***
6	0.31*	-0.19 NS	-0.49***	-144 NS	-391***
7	-0.47**	-0.20 NS	0.01 NS	-204 NS	7 NS
8	-0.19 NS	-0.05 NS	0.05 NS	- 46 NS	38 NS
9	-0.08 NS	0.10 NS	0.18 NS	112 NS	150 NS
10	-0.40***	-0.13 NS	0.03 NS	-141 NS	23 NS
MEAN	-0.08 NS	-0.09 NS	-0.04 NS	-106 NS	-35 NS

The correlations were weak and mostly not significant. An increase of 1 unit rust resistance corresponded with an annual loss in DM of 180 kg/ha (= 1.4 %) in series 1. The decline was limited to 0.6 % in the much more productive trials of series 2.

The yield of the rust cut was very close to 20 % of the annual yield in both series.

There was no significant correlation between the DMY and the rust resistance in this particular cut in series 1. We found a negative correlation in series 2 with an exception for the early types. Omitting the infected cut didn't change the results in series 1. Series 2 behaved differently : again with the exception of the earlies, the relationship between the yield of the healthy grass and the rust resistance was slightly positive.

On average the annual yield loss per unit increased rust resistance was 1 %. This makes a difference of 4 % between a very susceptible and a resistant variety which corresponds well with the results found by FEUERSTEIN *et al.*, 1994.

4. The relationship between the digestibility and the rust resistance in 1994 and 1995

Table 9 gives the coefficients of correlation (r) and regression (b) between the digestibility and the rust resistance.

With only 1 exception out of 7 trials we found a significant to highly significant positive correlation between the digestibility of the infected cut and the rust resistance. On average the digestibility improved with 1 unit per unit increase in rust resistance.

If one omits trial 7 this value becomes 1.5.

The relationship between the mean annual digestibility and the rust resistance remains positive but less significant.

One unit increase in rust resistance corresponded with about 0.3 units increase in digestibility.

If one omits the infected cut the relationship between the digestibility and the rust resistance was neutral.

Table 9 : Coefficients of correlation (r) and regression (b) between the digestibility (%) (A, B, C) and the crown rust resistance in yield trials during 1994
A : infected cut ; (B) : all cuts (weighted annual mean) , (C) : (B) - (A)

Trial	Number of families/ varieties	r			b		
		(A)	(B)	(C)	(A)	(B)	(C)
1	15	0.76***	0.54*	0.22 NS	1.89***	0.63*	0.21 NS
2	46	0.72***	0.35*	-0.12 NS	1.61***	0.31*	-0.12 NS
3	34	0.41*	0.09 NS	-0.09 NS	0.85*	0.12 NS	-0.13 NS
4	50	0.50***	0.21 NS	-0.15 NS	1.12***	0.19 NS	-0.12 NS
5	33	0.60***	0.35*	0.09 NS	1.84***	0.55*	0.12 NS
6	25	0.56**	0.39 NS	0.23 NS	1.39**	0.49 NS	0.27 NS
7	22	-0.38 NS	-0.27 NS	-0.12 NS	-1.21 NS	-0.38 NS	-0.17 NS
Total	225						
Mean							
1→7					1.07***	0.27***	0.01 NS
1→6					1.45***	0.38***	0.04 NS

In addition to the work in the yield trials we have analysed the digestibility of a series of diploid clones in October 1995. Our clonal field with over 2,000 clones was heavily infected with crown rust in that period.
5 clones with a very vigourous growth were chosen completely ad random for each rust class. The digestibility values ranged from 56 to 72 within this set of 25 clones.
The b-value for the linear-regression of the digestibility on the rust resistance equalled 1.9***. An improvement of 1 unit rust resistance corresponded with a 1.9 units increase of the digestibility. This result comes close to the results found in the yield trials.

5. The relationship between the artificial infections (AI) and the natural epidemics (NI)

Table 10 gives the coefficients of correlation between the NI and the AI. The correlation is positive significant but low. This is not very different from the results found thus far in this paper : all the correlations between assessments of the rust resistance in different circumstances (years, locations) were low and hardly higher than 0.50-0.60.
However the relationship AI - NI is worse than the relationships between different natural conditions (see 1.).
One of the best results is the result obtained in 1995. The probable reason is the use of Belgian spores instead of the pan-European mixture used in 1993 and 1994.

Table 10 : Coefficients of correlation between the rust resistance after NI (scored 1-5 ; 5 = no rust) and the AI in Merelbeke (% plants in the 2 best classes)

NI	Location	AI					
		1993		1994		1995	
		n	r	n	r	n	r
	B	118	0.43***				
1993	NL	101	0.31**				
	F	101	0.54***				
	B			115	0.32***		
1994	NL			120	0.21*		
	F			120	0.33***		
1995	B					102	0.67***

CONCLUSION

The heritability of the rust resistance is high, opening a good scope to improve the disease resistance. This optimism is hampered by the negative correlation with the yield. There is no decline in digestibility to expect. What bothered us the most is not the limits in the breeding but the constraints in the selection. The incidence of crown rust is very variable between years, locations and between spontaneous and artificial infections.

One has to pay the most attention to select the right genotypes. Observations during several years on several locations seem to be the best solution. But this is the general rule in breeding work.

REFERENCES

BIRCKENSTAEDT, E. 1990. Dissertation zur Erlangung des Grades Doktor der Agrarwissenschafter der Hohen Landwirtschaftlichen Fakultät der Rheinischen Friedrich-Wilhems-Universität, Bonn (D), 156 p. Entwicklung von Methoden für die Selektion auf Kronenrostresistenz bei Lolium spp. aus phytopathologischer Sicht.

CHOSSON, J.F. ; SINGLA, S. 1989. Sélection du ray-grass d'Italie pour la tolérance vis-à-vis de la rouille courronnée. XVI Congès International des Herbage, Nice (F) : 329-330.

FALCONER, D.S. 1981. Introduction to quantitative Genetics, Longuran, London & New York.

FEUERSTEIN, U. *et al.* 1994. Breeding for disease resistance in forage grasses with special emphases on 'cost of resistance'. IOBC/WPRS. Bulletin, 17 (1) : 13-19.

HAYWARD, M.D. 1977. Genetic Control of Resistance to Crown Rust (Puccinia coronata) in Lolium perenne L. and its Implications in Breeding. T.A.G., 51: 49-53

POSSELT, U. 1994. Genetic aspects of crown rust resistance in the ryegrasses. IOBC/WPRS. Bulletin, 17 (1) : 229-235.

REHEUL, D. ; GHESQUIERE, A. 1994a. Breeding ryegrasses with an improved Puccinia coronata resistance. Med. Fac. Landbouww. Univ. Gent, 59/3a : 971-976.

REHEUL, D. ; GHESQUIERE, A. 1994b. Experciences with artificial Puccinia coronata infections in ryegrass breeding. IOBC/WPRS. Bulletin, 17 (1) : 237-240.

SCHMIDT, D. 1980. La sélection du ray-grass d'Italie pour la résistance à la rouille couronnée. Rech. Agron. Suisse, 19 (1/2) : 71-84.

WILKINS, P.W. 1975. Inheritance of Resistance to Puccinia coronata Corda and Rhynchosporium orthosporum Caldwell in Italian Ryegrass. Euphytica, 24 : 191-196.

An artificial inoculation method to screen for resistance to *Fusarium*-rot in grasses

C.H.A. Snijders en G.D. Winkelhorst

DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, the Netherlands.

ABSTRACT

In the period September 1993 till May 1995, from several locations in the Netherlands 31 *Fusarium* isolates were isolated from diseased samples of turf grass, mainly perennial ryegrass (*Lolium perenne*) and red fescue (*Festuca rubra*). Of the *Fusarium*-isolates, 7 belonged to *F. culmorum* (W.G. Smith) Sacc., 4 to *F. graminearum* Schwabe, 3 to *F. avenaceum* (Fr.) Sacc., 2 to *F. acuminatum* Ell. & Ev. sensu Gordon and 15 to *F. crookwellense* Burgess, Nelson & Toussoun. *F. crookwellense* was dominating and not *F. nivale* (Fr.) Ces (snow mould) and *F. culmorum* as was expected. For inoculation experiments, *F. crookwellense* conidiospores were produced in a liquid SNA-medium. Sterile containers were filled with vermiculite and nutrient solution in which 400 seeds were sown. 22 days after sowing, plants were trimmed and inoculated by spraying a conidiospore suspension in three concentrations. Inoculation resulted in aerial mycelium coverage and necrosis of the turf. Inoculation with a conidiospore suspension of 200.000 spores/ml proved to be a rapid and discriminating method for resistance screening.

Keywords: Fusarium blight, turf grass, artificial inoculation, resistance

INTRODUCTION

Persistency of amenity grasses under a close cutting regime in the wet and cool climate of north-western Europe depends for a large part on resistance to diseases, of which *Fusarium* is particular important. In turf grasses, *Fusarium*-damage includes seedling blight, crown and root rot, patch disease and snow mould. Dead patches as a result of

blight, crown and root rot, patch disease and snow mould. Dead patches as a result of crown- and root rot usually do not regenerate and are replaced up by undesired grasses like *Poa annua* and weeds. This leads to quality reduction of the turf. Several *Fusarium* species may cause this disease. In cold winters, *F. nivale* (syn. *Gerlachia nivalis* (Ces.ex Sacc.) = *Microdochium nivale* (Fr.) Samuels & Hallet), snow mould, in general dominates (Smiley *et al.* 1994). *Fusarium* is both seed- and soil-borne.

In grass breeding programmes, selection for resistance to *Fusarium*-rot depends on natural epidemics which occur erratic in time and over locations. Therefore, in a collaboration programme between Dutch grass breeding companies (Barenbrug Holland BV, Cebeco Zaden NV, J. Joordens Zaadhandel, Limagrain Genetics, Mommersteeg International BV, Zelder BV) and CPRO-DLO, an efficient and reliable artificial inoculation method is developed which screens plants in seedling boxes. To get an impression of the causal *Fusarium*-pathogen in the Netherlands, during two years *Fusarium*-infections were monitored for species frequency.

MATERIALS AND METHODS

In the period September 1993 till May 1995, from locations in the Netherlands *Fusarium* was isolated from diseased samples of turf grasses, and from infected seed. Diseased leaves and roots were plated on PDA and SNA-medium (Nirenberg 1981). Emerging colonies were subcultured on both PDA- and SNA-medium. PDA-medium was used to study the colony morphology, SNA was used to study conidospore morphology. *Fusarium* species were identified according to Nelson *et al.* (1983). All isolates were monospored and stored in cryopreservation fluid using the Protect bead storage system (Technical Services Limited, Heywood, UK). These monospore cultures form the start for inoculum production.

For the inoculation experiment, *F. crookwellense* (CPRO 94-408) conidiospores were produced in a liquid SNA-medium in 2 L Erlenmeyers which were aerated with filter-sterilized (Whatman HEPA-VENT L#5243) air for seven days.

Seeds of two *Lolium perenne* varieties ('Blazer', 'Talgo') and two *Festuca rubra* varieties ('Lobi', 'Rufilla') were superficially sterilized in a 10% Natrium Hypochlorite solution for 30 min. 20´7´7 cm sterile containers (Magenta, Sigma) were filled with vermiculite and Steiner's nutrient solution (Steiner 1984) and 400 seeds were sown. The containers were incubated in the phytotron under a temperature regime of 17/6°C, and 50 Watt/m^2 artificial

light during 15 h. The plants were regular trimmed to stimulate turf formation. Immediately after the third cutting, 22 days after sowing, plants were inoculated by spraying a conidiospore suspension till run-off, for which 2.5 ml of an inoculum suspension was used per container. The inoculation was applied in three concentrations: 20.000 spores/ml, 200.000 spores/ml and 2.000.000 spores/ml. The control was sprayed with sterile water. The experiment was carried out in four replicates.

Table 1. *Fusarium* isolated from diseased samples of turf and sportgrasses

Collection number	*Fusarium* species	isolation substrate	location	isolation date
CPRO 93-31	*acuminatum*	*Festuca rubra*	Kessel	10-9-1993
CPRO 95-011	*acuminatum*	*Poa pratensis*	Kessel	12-7-1995
CPRO 93-55-1	*avenaceum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-61	*avenaceum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-9	*avenaceum*	grass	Papendal	2-9-1993
CPRO 93-26	*crookwellense*	*Festuca longifolia*	Wolfheze	15-9-1993
CPRO 93-32	*crookwellense*	*Festuca rubra*	Kessel	10-9-1993
CPRO 93-46	*crookwellense*	*Festuca longifolia*	Wolfheze	15-9-1993
CPRO 93-50L	*crookwellense*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-59b	*crookwellense*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-60-2	*crookwellense*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-61-1	*crookwellense*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-7	*crookwellense*	grass	Papendal	2-9-1993
CPRO 93-8L	*crookwellense*	grass	Papendal	2-9-1993
CPRO 93-8R	*crookwellense*	grass	Papendal	2-9-1993
CPRO 94-408	*crookwellense*	*Lolium perenne*	Gennep	27-9-1994
CPRO 95-003	*crookwellense*	*Festuca rubra 'Rufilla'seed*	CPRO-DLO	13-3-1995
CPRO 95-009	*crookwellense*	*Festuca rubra* 'Lobi' seed	CPRO-DLO	13-3-1995
CPRO 95-010	*crookwellense*	*Festuca rubra* 'Rufilla' seed	CPRO-DLO	13-3-1995
CPRO 93-28	*crookwellense* atypical	*Festuca longifolia*	Wolfheze	15-9-1993
CPRO 93-1	*culmorum*	grass	Papendal	2-9-1993
CPRO 93-50R	*culmorum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-51	*culmorum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-55-2	*culmorum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-60-1	*culmorum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 95-008	*culmorum*	*Lolium perenne* 'Talgo' seed	CPRO-DLO	13-3-1995
CPRO 95-013	*culmorum*	*Lolium perenne* seed	unknown	1995
CPRO 93-56	*graminearum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-59a	*graminearum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 93-61-2	*graminearum*	*Lolium perenne*	Vlijmen	24-9-1993
CPRO 95-014	*graminearum*	*Agrostis capillaris* stem	Wolfheze	1995

RESULTS

In the Netherlands, diseased grass samples were collected from the breeding stations of Joordens Zaadhandel in Kessel, Mommersteeg-Van der Have in Vlijmen, CEBECO Zaden in Vlijmen, Nederlandse Sport Federatie in Papendal near Arnhem, Zelder in Ottersum near Gennep, and Barenbrug in Wolfheze. Also some *Fusarium*-isolates were collected from infected seed. A total of 31 *Fusarium* isolates were isolated from diseased samples of perennial ryegrass (*Lolium perenne*), red fescue (*Festuca rubra*), smooth-stalked meadowgrass (*Poa pratensis*), *Festuca pratensis* and *Agrostis capillaris* (Table 1). *Fusarium nivale* was not isolated from any of the diseased samples from the Netherlands. From our data the conclusion can be made that in the period September 1993 - November 1995 *Fusarium* blight, either in winter or summer, was not caused by *F. nivale*, but predominantly by *F. crookwellense*.

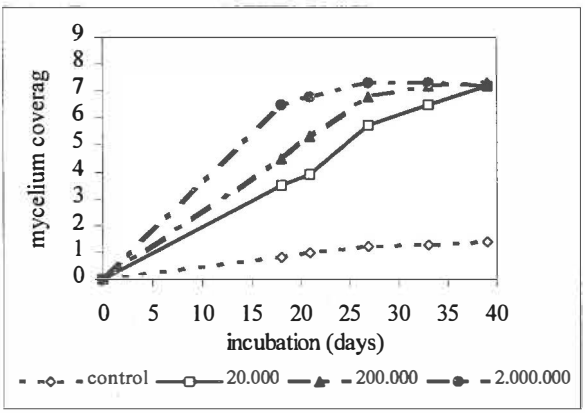


Figure1. Development of aerial mycelium on turf in time

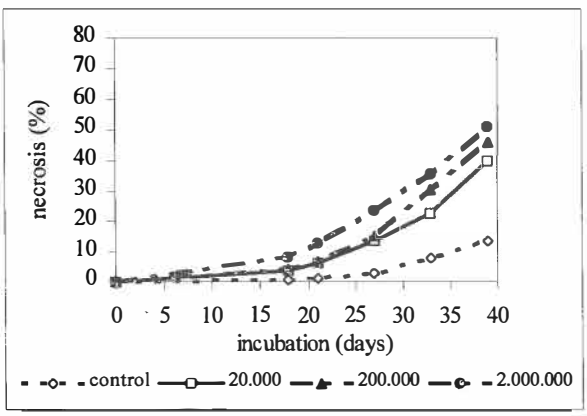


Figure 2. Development of necrosis of the turf in time

The inoculation of the turf in the containers resulted in aerial mycelium development. The mycelium was plated on PDA and identified as *Fusarium crook-wellense*. At 18 days after inoculation, mycelium covered part of the turf and first observations were made. Mycelium coverage was observed at regular intervals (Figure 1) on a 0-9 scale where 9 indicates that the turf was completely covered by the

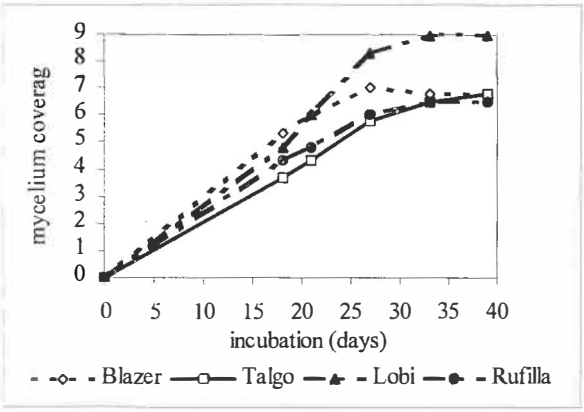


Figure 3. Development of aerial mycelium on turf in time

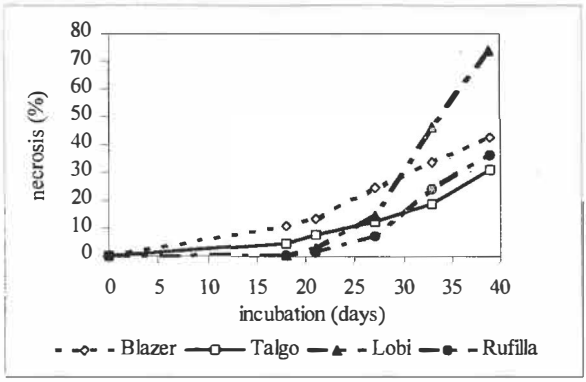


Figure 4. Development of necrosis of the turf in time

mycelium. Besides mycelium coverage also necrosis was observed as a percentage of the turf killed by the fungus (Figure 2). All three inoculum concentrations led to mycelium coverage and necrosis. The higher the concentration, the higher the growth rate of the mycelium during the first 20 days. The necrosis is ranked according to inoculum concentration. Also in the control objects mycelium emerged which was identified as *F. crookwellense* or *F. culmorum* (Table 1).

Statistical analysis (not shown) and Figures 1 and 2 show that there is no interaction between inoculum concentration and incubation time. Therefore the reactions of the four turfgrass varieties are only analyzed for the inoculation with 200.000 spores/ml. Figures 3 and 4 show the development of mycelium coverage and necrosis for the four varieties. Statistical analysis (not shown) and Figures 3 and 4 show that there is an interaction between incubation time and variety. This interaction is caused by the variety 'Lobi' which initially has a low rate of mycelium coverage and necrosis, but after 25 days develops as the most susceptible variety.

DISCUSSION

Schumann *et al.* (1987) showed that in summer 17% of symptomless plants of Italian ryegrass (*Lolium multiflorum*) at the stem base were infected by *Fusarium*, of which

12% by the pathogenic species *F. culmorum*, *F. graminearum* and *F. nivale*. Mycological analysis of necrotic leaves of *Lolium perenne* and *Lolium multiflorum* showed in 81% of the samples *Fusarium* of which 34% for *Lolium* pathogenic species (Engels & Krämer 1994). *F. crookwellense* was first identified in 1982 (Burgess 1982) and first described in the taxonomic system of Nelson *et al.* (1983). It is striking that Schumann *et al.* (1987) and Engels & Krämer (1994) did not identify *F. crookwellense*. This may be caused by the fact that the taxonomic system they used did not recognize this species.

Species identification of *Fusarium* is difficult. Conidiospores of *F. culmorum* are morphologically close to *F. crookwellense*. Atypical spores of *F. crookwellense* can easily be confounded with *F. graminearum*. Therefore a molecular marker system is now being developed to serve as an extra tool for species identification, which may lead to reclassification of some of the isolates in Table 1.

Seed-borne infection is a problem in artificial inoculation tests. At this time a warm-water treatment is optimized to kill *Fusarium* in the seed. Seed-borne infection is an important cause of seedling blight and forms the starting inoculum for *Fusarium* blight.

Mycelium coverage indicates that *Fusarium* is growing on the turf. Necrosis indicates that the plant is killed. Necrosis is what causes crown and root rot in the field.

According to the Dutch Recommended List of Varieties 1996 which uses a scale of 0-9 (9 is completely resistant) for foot rot resistance based on field data, 'Lobi' scores 7.5, while 'Rufilla' only 6. Our inoculation test could not confirm the slightly higher resistance level of 'Lobi'.

REFERENCES

Burgess, L.W., P.E. Nelson & T.A. Tousson, 1982. Characterization, geographic distribution and ecology of *Fusarium crookwellense* sp.nov. Trans.Br.Mycol.Soc. 79: 497-505.

Engels, S.R., & J. Krämer, 1994. Incidence of Fusaria on Italian and perennial ryegrass *(Lolium multiflorum* Lam. and *Lolium perenne* L.). International Conference on Harmful and Benificial Microorganisms in Grassland, Pastures and Turf. Ed.: K. Krohn, V.H. Paul, J. Thomas. IOBC wprs Bulletin 17(1): 59-64.

Nelson, P.E., T.A. Toussoun & W.F.O. Marasas, 1983. *Fusarium* species: An illustrated manual for identification. University Park & London. The Pennsylvania State University Press. 193pp.

Nirenberg, M., 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Can.J.Bot. 59: 1599-1609

Schumann, K. C. Janke & C. Wolf, 1987. Untersuchungen zum Halmbefall des Welschen Weidelgrases durch *Fusarium*-Arten. Nachrichtenblatt für den Pflanzenschutz in der DDR 41: 164-167.

Smith, J.D., N. Jackson & A.R. Woolhouse, 1989. Fungal diseases of amenity turf grasses. E. & F.N. Spon, New York. 401 pp.

Steiner, A.A., 1984. The universal nutrient solution. In: Proceedings of the VI International Congress on Soilless Culture, Lunteren, The Netherlands 1984. pp. 663-650.

Assessment of selection for tolerance to bacterial wilt in italian ryegrass

Michele Betin - Regine Samson* - Jean Gondran & Marc Ghesquiere

Station d'Amelioration des Plantes Fourrageres - INRA
F-86600 LUSIGNAN (FRANCE)
* Station de Pathologie Vegetale et Phytobacteriologie - INRA
42, Rue Georges Morel - BP 57
F-49071 BEAUCOUZE CEDEX (FRANCE)

Most of forage grass species are highly susceptible to *Xanthomonas campestris*, a mucous vascular bacteria inducing rapid wilt of infested grasses at heading time, eventually up to death when temperature is hot. As breeding for more tolerant varieties is only conceivable, we have developed a procedure of mass selection within family based on artificial inoculation under controlled environment. We reported hereafter the genetic progress recorded from 3 generations of selection within a diploid and a tetraploid population of Italian ryegrass.

Material and methods

Both populations of Italian ryegrass, highly persistent and tolerant to rust (*P. coronata*), were derived from polycrosses of 88 tetraploid individuals and 68 diploid ones resp. Half-sibs families of each population underwent alternately 3 generations of assessment and selection. Each year during Summer, 4- to 5-week old seedlings of either population were inoculated in greenhouse by clipping individually the plants with scissors previously dipped into a water solution of bacteria concentrated at a rate of 10**8 / ml. The strain of *X. campestris* pv *graminis* which was used for any annual assessment was at first selected from a preliminary test of pathogenicity of different strains on 3 control Cvs:
'Adret': susceptible - 'Turilo': intermediate - 'Lipo': tolerant.

A Four-block trial was used each year with 80 individuals per family except at the last generation of the diploid population where the design was a 10 x 9 x 10 balanced lattice

with 100 individuals inoculated per family. From 1 to 13 weeks following inoculation, symptoms of wilt were individually recorded. When they were fully expressed, individuals having no more than one wilt tiller or only white striped leaves (which is hold as a reaction of tolerance) were retained and allowed to be seed multiplied next year in isolated conditions.

Both populations were selected following basically the same procedure except that the number of individuals which were retained within each family for next generation varied widely among families and generations depending on the level of symptoms reached on average in the annual trials. Moreover, 39 highly susceptible tetraploid families were at first discarded while a global mass selection within the tetraploid population followed by polycrossing gave 16 new families which were then selected in the same way as the two other populations up to the third generation.

The rate of mortality within family was weekly recorded and was used to assess the overall genetic progress of selection for tolerance over generations. Unbalanced nested model of analysis of variance was applied for computing the whole data set by retaining from each annual trial the rate of mortality recorded at a date when it was the most discriminating between the three constant control Cvs.

Results

The rate of mortality which was recorded from 623 families and controls over 8 years showed that tolerance in the last generation of selection within either ploidy reached the level of the Cv 'Turilo' (Fig. 1). In some progenies, inoculated plants were found with no wilt symptom and least-squares mean of the rate of mortality within the best families was even close to zero although not significantly different from the Cv 'Lipo' because of high error standard deviation (15 %). The overall generation effect was only significant from the G0 to G1 generation; among populations, only the tetraploid population derived from mass selection and polycrossing was significantly different from the two other ones at the G2-generation (Tab. 1). No change in phenotypic variance between families within either population occurred over generations (Fig. 2).

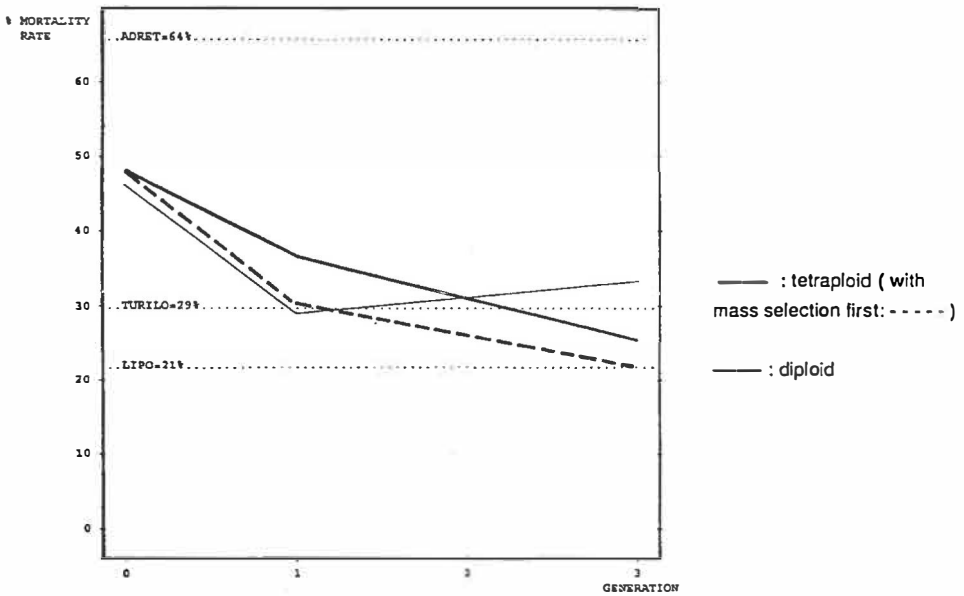


Fig. 1: Average rate of mortality over 3 generations of selection within diploid and tetraploid populations of italian ryegrass

Rate of mortality:

	G0	G1	G2	G3
Tetraploid level				
49 ———	48.9%	34.1%	33.4%	22.9%
16 - - - - - - - -		30.9%	25.2%	24.2%
Diploid level				
68 ———	46.5%	27.2%	32.1%	31.9%

Intensity of selection:

	G0	G1	G2	G3
Tetraploid level				
49 ———	11.7%	32.9%	21.9%	
16 - - - - - - - -	0.2%	33.2%	20.1%	
Diploid level				
68 ———	21.2%	18.6%	27.3%	

Tab. 1: Rate of mortality and intensity of selection within family

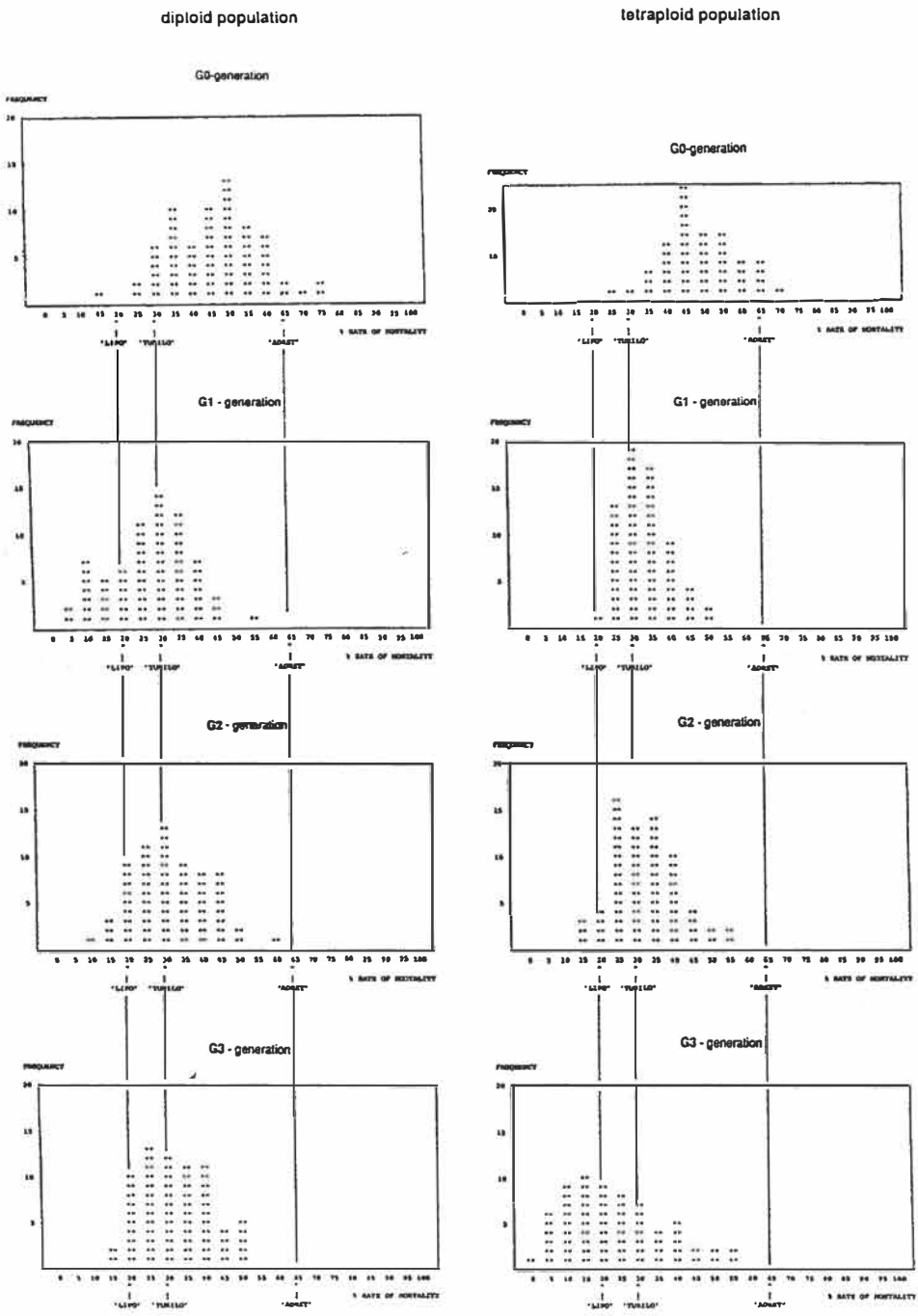


Fig. 2: Distribution of the family least-squares means of mortality rate

Discussion

On average, most of the genetic gain was achieved after the first generation of selection halving the rate of mortality within family. The tetraploid population appeared to be more susceptible than the diploid population since 39 families had to be at first discarded. Then, only a high rate of mass selection within the tetraploid population and polycrossing allowed to reach the level of tolerance of the diploid population. In the next generations, the tetraploid populations seemed to have responded to selection whereas the diploid population did not or even appeared to have drifted towards more susceptibility. In fact, the model of analysis of variance we used assumed no genotype x year interactions when we know that they likely occurred. For instance, symptoms on plant were particularly light some year and did not allow to actually select among populations with the same intensity from one generation to the other. Also, selecting every year the most pathogenic strain of *X. campestris* to be inoculated to insure a similar level of symptoms over trial could have led, on the contrary, to increase unintentionally already large year x genotype interactions.

In a first approach, the genetic control of tolerance appeared to be additive when comparing the response of the diploid vs tetraploid population. Several genes with complementary effects could be also involved as polycrossing plants selected from different families was particularly effective to decrease susceptibility within the tetraploid population. However, so many uncontrolled effects were expected to have been confounded and to have given apparently quantitative variations that it is difficult to conclude whether tolerance is actually polygenic or controlled by only few genes.

To improve further bacterial wilt tolerance, we conclude that a selection based on progeny tests could complete more definitely the progress achieved following first generation of mass selection. Such a selection would allow to establish whether tolerance in Cvs such as 'Lipo' can be gone beyond or if it represents a maximum to be not overcome given the current conditions of assessment. Finally, controlling more acurately the environmental conditions during the assessment and selection process as well as the variability in pathogenicity of *X. campestris* strains should also greatly contribute to make selection for tolerance more effective in advanced generations.

Crop Protection and Environment

New aspects concerning biological control of *Cirsium arvense*

S. Guske, C. Boyle and B. Schulz

Institut für Mikrobiologie, TU Braunschweig, Spielmannstr. 7, 38106 Braunschweig, Germany

ABSTRACT

Our present goal for biocontrol of the Canadian thistle, *Cirsium arvense,* has been to isolate a host specific necrotrophic fungus that can be applied singularly or in combination with another host specific fungus (double infection). Following surface sterilisation, 890 necrotrophic and endophytic fungi were isolated as potential primary or secondary invaders from diseased and healthy plants of *Cirsium arvense.* While the same spectrum of fungal genera was found in healthy and diseased plants, the proportion of phytopathogenic isolates was higher in the diseased plants.

Aggressiveness of 543 of the isolates was tested using the alga, *Chlorella fusca*, and the thistle in leaf segment tests. Endophytes, as a group, had the highest proportion of isolates showing algicidal (herbicidal) activity, while isolates from diseased thistles were more aggressive in the leaf segment tests than the endophytes were. The 47 isolates with the ability to cause disease on thistle segments were checked for host specificity using leaf segments of plants of the surrounding flora as well as using plants of common agricultural crops.

At the end of the screening four promising isolates were chosen for subsequent testing on whole plants in growth chambers. The two most aggressive and specific isolates were *Phoma destructiva* and *Phoma dennisii.* They seem suitable for further experiments in developing a mycoherbicide.

Infection with *P. destruktiva* had been found to be responsible for the many chlorotic thistles found in a pasture near Wunstorf, Lower Saxony. *In vitro*, it was only possible to attain chlorotic thistles following a systemic infection of the roots with *P. destruktiva.*

The strategy of using a double infection was also investigated. By combining the biotrophic rust, *Puccinia punctiformis,* with one of the necrotrophic or endophytic isolates, the intensity of the infection symptoms could be increased.

Keywords: *Cirsium arvense*, biological weed control, necrotroph, endophyte

INTRODUCTION

Cirsium arvense, the Canadian thistle, is a wide-spread weed of pastures, fallow and arable land (Hayden, 1934). Especially due to the recent increase in land that is being laid fallow, the thistle has become more and more of a problem. It primarily spreads via its extended root system which develops at a depth of up to one meter. Herbicides are not well suited for controlling *C. arvense*, because of the development of resistances, possible effects on other dicotyledonous culture plants (Hagger, 1986) and side effects on the ecosystem. Additionally, herbicides don't always reach all of the underground roots.

Thus, biological control seems a sensible alternative. Logical candidates for biocontrol are host specific fungi, for example the thistle rust, *Puccinia punctiformis* and/or necrotrophic pathogens or endophytes. However, in one respect, use of the rust as a biocontrol agent for *C. arvense* is problematic. Only a systemic, as opposed to a local, infection with *P. punctiformis* is able to kill a thistle plant. Other approaches to biological control of the Canada thistle might include the following strategies:

1) **single infection**
- specific necrotroph/endophyte

2) **double infection**
- local rust infection + unspecific necrotroph/endophyte
- local rust infection + specific necrotroph/endophyte

3) **infection + toxin**
- local rust/necrotroph/endophyte infection + specific toxin

METHODS:

890 fungi were isolated from leaf, stem and root segments (30 per organ and plant and time) following surface sterilisation (hypochloric acid) from: systemically rust-infected plants, macerated thistles, thistles with large chlorotic areas which later turned brown and black (pasture near Wunstorf, Lower Saxony) and endophytes from healthy thistle plants. Endophytes were also isolated, because endophytic fungi may be latent pathogens and may also show a high degree of specificity (e.g. Leuchtmann & Clay, 1993; Lu & Clay, 1994; Toti et al., 1992).

To find isolates with algicidal activity, 543 of the above isolates were screened using the test organism ,*Chlorella fusca,* in agar diffusion tests with growing fungi. In our laboratory we have found that algicidal activity generally correlates with herbicidal activity (unpublished results). For comparison of algicidal activity the activity number A was introduced. This number varies from 0 (no inhibition) to 4.2 (complete growth inhibition) (1). It enables the direct comparison of a small but total inhibition with a large, but not total, inhibition. Those fungi, which inhibited *Chlorella* were tested on thistle leaf segments. Intact and perforated segments were placed on water agar and sprayed with a spore or mycelial suspension. To compare the destructiveness of spore or mycelial suspensions of selected isolates, a destructive number was introduced to evaluate the symptons (2). It can assume values between 0 and 30, with 0 = 0% disease. The 47 isolates with the ability to cause disease on thistle segments were checked for host specificity against wheat, sugar beet, rape and dandelion. At the end of the screening four promising isolates were chosen for subsequent testing under climatically controlled conditions in growth chambers on thistles as well as on wheat, sugar beet, rape and dandelion.

Calculation of the inhibition activity (A) of fungi in agar diffusion tests
(according to Marquarding, 1992)

activity number $A = \sqrt{x \cdot y}$ with: **(1)**

- x = value and type of inhibition
  - 0 = no inhibition
  - 1 = low growth inhibition (±gi)
  - 2 = considerable growth inhibition (gi)
  - 5 = complete growth inhibition (cgi)
- y = radius of growth inhibition 0 to 3.5

examples:

	inhibition	radius	calculation	activity number A
isolate 1	cgi	2 cm	$A = \sqrt{5 \cdot 2}$	= 3.16
isolate 2	gi	2.5 cm	$A = \sqrt{2 \cdot 2{,}5}$	= 2.24
isolate 3	cgi + gi	0.3 cm + 0.8 cm	$A = \sqrt{A_I} + \sqrt{A_{II}}$	
			$A = \sqrt{5 \cdot 0{,}3} + \sqrt{2 \cdot 0{,}8}$	= 2.49

Calculation of the destructive activity of fungi in leaf segment tests (D)
(according to Guske, 1995)

destructive number $D = \sum S - \sum K$ with: **(2)**

- S = type of symptom
  - **n**ecrosis n% ⇒ n : 10 3 = S_1
  - **m**acerated m% ⇒ m : 10 2 = S_2
  - **c**hlorotic c% ⇒ c : 10 1 = S_3
- K = type of variation of the water control segments
  - **n**ecrosis n% ⇒ n : 10 3 = K_1
  - **m**acerated m% ⇒ m : 10 2 = K_2
  - **c**hlorotic c% ⇒ c : 10 1 = K_3

examples:

	type of symptoms	calculation	destructive number D
isolate 1	10% n + 20% m + 15% c	S_1 = 10 : 10 3 = 3 S_2 = 20 : 10 2 = 4 S_3 = 15 : 10 1 = 1.5	$D = S_1 + S_2 + S_3 - K_1 + K_2$
control	5% n + 10% c	K_1 = 5 : 10 3 = 1.5 K_2 = 10 : 10 1 = 1	D = 6

RESULTS

The procentual distribution of the most commonly isolated genera from the leaves, stems and roots is shown in Tab. 1. One striking result is that species of the genus, *Alternaria*, frequently isolated as pathogens, saprophytes or endophytes, were isolated only from a relatively small proportion of the healthy tissues. On the other hand, 24.3% of the isolates from macerated tissue belonged to the genus *Alternaria*.

Yeasts and isolates of the genus, *Aureobasidium,* seem to play an important role in the stems of healthy plants. 14% of the total isolates from stems were yeasts. In our laboratory we have found a comparable distribution and proportion of yeast isolates in clover and parsley. In diseased thistles, this ratio changed. In the case of *Aureobasidium*, we found a ten fold reduction in the proportion of *Aureobasidium* isolates from diseased as compared to healthy tissue.

Phoma destruktiva was the species most frequentley isolated from the leaves and stems of chlorotic thistle plants. None of the other fungal strains isolated was able to invade the chlorotically weakened tissue of leaves and stems with an infection density comparable to

those of *P. destruktiva*. In healthy plants, *P.destruktiva* was encountered much less frequently.

Although the endophytes were isolated from asypmtomatic tissue, both the endophytic fungal isolates and the isolates from diseased tissue belong to common saprophytic genera: *Alternaria, Aureobasidium, Cladosporium, Penicillium, Phoma and Fusarium.*

Table 1: Comparison of the distribution of the most common fungal isolates in percent of all segments tested (30 segments/organ and plant, five locations, Lower Saxony, FRG, summer 1994) and based on 890 isolates from surface sterilised thistle segments;
example: leaf-*Alternaria*: 5.8 = 5.8% from the isolates of healthy plants

organ	isolate	healthy	systemically rust-infected	chlorotic	macerated
	Alternaria				
leaf		**5.8**	5.6	1.2	**24.3**
stalk		**7.6**	1.9	1.2	3.5
root		0	0	3.0	n.t.
	Aureobasidium				
leaf		0.3	3.1	0	7.1
stalk		**9.8**	1.3	0.6	1.8
root		0.9	1.9	0.6	n.t
	yeasts				
leaf		7.3	4.4	0.6	0
stalk		**13.7**	6.3	4.2	4.4
root		6.7	6.3	1.8	n.t.
	Cladosporium				
leaf		2.4	5.6	0	0.9
stalk		0.6	0	0.6	3.5
root		0.9	0	0.6	n.t.
	Fusarium				
leaf		0.3	0.6	0	3.5
stalk		0	6.3	0	1.8
root		0.3	5.6	3.6	n.t.
	Phoma				
leaf		2.7°	0.6	**27.0***	4.4
stalk		5.5°	0.6	**22.8***	6.2
root		3.1°	0	1.2*	n.t.

Strains of the following genera were isolated less frequentley:
Acremonium, Artrinium, Chaetomium, Ciferiella, Coniothyrium, Cylindrocarpon, Colletotrichum, Dendryphion, Dictyostelium, Epicoccum, Exophiala, Geomyces, Gliocladium, Humicola, Mycelia sterila, Paecilomyces, Penicillium, Phomopsis, Phlyctema, Polyscytalum, Torula, Tricellula, Trichoderma, Rhizoctonia, Sordaria, Stemphylium

To ascertain whether or not the isolates produce substances that are biologically active, and in particular herbicidally active, a screening was done using the test organism, *Chlorella fusca*. 543 of the 890 necrotrophic and endophytic fungi were tested in such a screening. We compared algicidal activity with the source of the isolates (Fig. 1). The best source of algicidally and probably herbicidally active isolates is the healthy thistle plant. 38% of the endophytes tested inhibited *C. fusca*. The next best source is tissue systemically infected with rust, followed by chlorotic and finally macerated thistles.

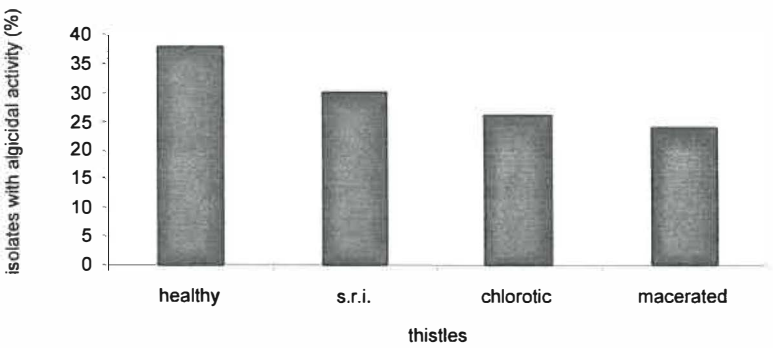


Fig. 1: Proportion of isolates with algicidal activity from healthy, systemically rust infected and macerated thistles. s.r.i. = systemically rust infected

Since it could be assumed that algicidal and herbicidal activity correlate, those isolates that had inhibited *Chlorella* were selected for leaf segment tests using the host. Healthy plants harbour the smallest proportion of isolates effective against the thistle, the maximum being 15% (Fig. 2). From diseased thistles 20 - 25% of the isolates were active against the thistle. Our results comparing perforated and non-perforated leaves showed

that the extent of necrosis was often higher on the leaves that had been previously wounded. Leaf segment tests are an effective manner of simulating wounded leaves, as would be the case in a double infection using a rust infection as an initial host-specific pathogen.

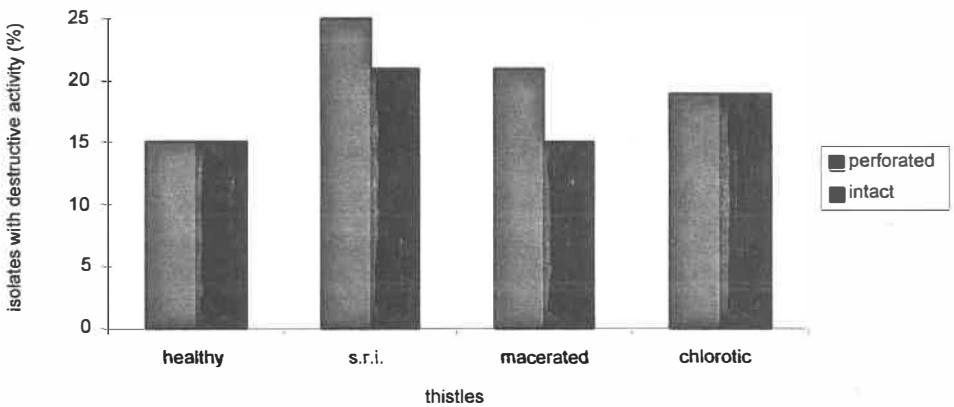


Fig. 2: Leaf segment tests of the host (*Cirsium arvense*) on perforated and intact segments using isolates from healthy, systemically rust infected and macerated thistles based on the destructive number (D). The data represent all isolates with $D > 7$ ($D_{max} = 30$).

In addition to determining the aggressiveness of the isolates towards the thistle, the specificity of the aggressive strains was established.

On the basis of their specificity and destructiveness, four fungi were chosen for experiments on whole plants (Tab. 2). These fungi were identified as *Phoma destruktiva, Phoma denisii, Fusarium tabacinum* and *Gliocladium roseum.*

Table 2: Leaf segment test for host specificity. Evaluation based on destructive activity (D, with $D_{max} = 30$) after 7/9 days.

fungal isolate	thistle	wheat	dandelion	sugar beet	rape
Fusarium tabacinum	22.5/27.5	4.5/4.0	0/1.5	2,5/2	1.5/1.5
Phoma dennisii	21/28.5	1.75/-5.5	4.5/5.5	1.5/-0.25	1.5/2.0
Phoma destruktiva	8.5/10.5	1.0/-1.5	0/0	1.5/1.25	1.5/1.5
Gliocladium roseum	1.8/6.5	-2.0/0	0/-1.5	-0.5/0	0/0.5

In all these investigations, we should bear in mind that the isolates of a certain fungal species can exhibit considerable phenotypic variation. Consequently, the range of reactions of the host plants to infection by a particular species can also vary considerably and depends on the particular isolate being tested (Tab. 3). The *Fusarium tabacinum* isolates differ greatly in their ability to cause disease, demonstrating this variability of individual strains. For example, following infection with 7 different strains, the reaction of the thistle leaf segments ranged from 4.5 to 25.5. To complicate the situation the variability of the fungal strains also resulted in different reactions of the leaf segments of plants of the surrounding flora. Additionally there was variability among the individuals of one plant species. This variablity is an essential point to be borne in mind when screening fungi for biocontrol.

In addition, the data show that algicidal activity does not necessarily correlate with specific aggressiveness against the thistle. For example, strain 12 did not inhibit growth of *Chlorella,* but it did severely damage the thistle in leaf segment tests.

Table. 3: Comparison of herbicidal activity of the fungus, *Fusarium tabacinum,* using an alga (*Chlorella fusca*) and leaf segments of the host

A = activity number, D = destructive number

Fusarium tabacinum Isolates	*Chlorella*-Test A ($A_{max.}$= 4.2)	thistle leaf segment test D ($D_{max.}$= 30)	
		perforated	intact
10IIIWG	0	20.5	7
12IIIWG	0	14.5	14.5
124IIWG	1.6	22.5	25.5
250IISR	0.4	10.5	12.5
251IISR	0.4	19	14
253IIWG	0.5	7.5	9.5
265IIIWR	2.0	4.5	6

Fusarium tabacinum, Phoma dennisii, Phoma destructiva and Gliocladium roseum (Tab. 2) were also tested using whole plants under climatically controlled conditions. Although after leaf application none of the isolates totally killed the test plants, the fungi, *Phoma dennisii* and *Phoma destruktiva,* showed the best results with respect to specificity and aggressiveness and thus seem suitable for further experiments to develop a mycoherbicide. In an attempt to attain a more damaging infection we used a soil application of spores of *P. destruktiva*. And, indeed, a soil application did result in a systemic infection and more destructiveness.

CONCLUSIONS

Plant pathogens continue to be potent weapons for biological control of problematic weeds (e.g. TeBeest, 1990, 1993; Charudattan, 1991). Preferably, the pathogens should be host specific and native organisms. Both our previous work using one obligate biotroph (*C.arvense/P.punctiformis)* (Völker & Boyle, 1994), and that using a double infection (*C.arvense/P.punctiformis* + necrotroph) (Boyle & Schulz, 1994) and the present work using host-specific necrotrophs follow these principles. With respect to the use of a necrotrophic isolate as a biocontrol agent, two isolates of the genus *Phoma*

were studied that seem suitable for development as mycoherbicides. The next important task will be optimizing their formulation and application.

REFERENCES

BOYLE, C. & SCHULZ, B. (1994): Alternative biologische Bekämpfungsstrategien gegen die Ackerkratzdistel. Z. PflKrankh. Pfl. Schutz, Sonderh. XIV, 243-252.

CHARUDATTAN, R. (1991): The mycoherbicide approach with plant pathogens. In: Microbial control of weeds. (ed. Te Beest, D.O.), Chapman and Hall, New York/London, 24-57.

HAGGAR, R.J., OSWALD, A.K. and RICHARDSON, W.G. (1986): Effect of herbicides on thistle rust. Crop Protection 5, 73-75.

HAYDEN, A. (1934): Distribution and reproduction of Canada Thistle in Iowa. Am. J. Bot. 21, 142-144.

LEUCHTMANN, A. and CLAY, K. (1993): Nonreciprocal compatibility between *Epichloë typhina* and four host grasses. Mycologia, 85, 157-163.

LU, M. & CLAY,K. (1994): Differential growth of *Atkinsonella* species on host grass calli. Mycologia, 86, 667-673.

TE BEEST, D.O. (1990): Ecology and epidemiology of fungal pathogens. In: Microbial control of weeds. (ed. Te Beest, D.O.), Chapman and Hall, 97-114.

TE BEEST, D.O. (1993): Biological control of weeds with fungal pathogens. In: Exploitation of Microorganisms (ed. Jones, D.G.), Chapman and Hall, 97-114.

TOTI, L., CHAPELA, I.H. & PETRINI, O. (1992): Morphometric evidence for host-specific strain formation in Discula umbrinella. Mycological Research, 96, 420-424.

VÖLKER, K. & BOYLE, C. (1994): Bean rust as a model system to evaluate efficiency of teliospore induction, especially in the potential mycoherbicide *Puccinia punctiformis*. Weed Research, 34: 275-281.

Biological control of insect pests in grasses

H.J.Vlug and P.H.Smits

Research Institute for Plant Protection (IPO-DLO)
Wageningen, The Netherlands

Grassland, both pastures, sportfields and amenity grassland is an important crop in The Netherlands. In many cases it is damaged by a variety of insect larvae. The species composition differs from use:

Pastures:

Tipulid larvae (leatherjackets, Wiesenschnaken): (Tipula paludosa, T. oleracea, T. subcunctans (=T. czizeki).

Bibionid larvae: Dilophus febrilis (fever fly).

Fritfly larvae: Oscinella frit (frit fly).

Scarabaeid larvae (white grubs, Engerlingen): Aphodius contaminatus (dung beetle), Aphodius spp. (dung beetles), Melolontha melolontha (may beetle, Maikäfer), Phyllopertha horticola (garden chafer).

Noctuid larvae (Lepidoptera).

Sportfields and amenity grassland:

Tipulid larvae (leatherjackets, Wiesenschnaken): Tipula paludosa, T. oleracea. Scarabaeid larvae (white grubs, Engerlingen): Aphodius spp. (dung beetles), Melolontha melolontha (may beetle, Maikäfer), Phyllopertha horticola (garden chafer), Amphimallon solstitiale (june beetle), Serica brunnea, Anomala dubia (A. aenea). Noctuid larvae (Lepidoptera)

Biological control

Biological control of white grubs with parasitic nematodes has been studied in The Netherlands for the last five years. The results from field experiments are as yet difficult to interpret. Some good results have been obtained and bioassays with different species of nematodes give good results. Penetration and survival of the nematods through the different types of thatch and soil are in being studied. During studies on the biology of the garden chafer the parasite Tiphia femorata (F.) was found in large numbers. In the early years of our study these parasites remained unnoticed despite intensive monitoring of the host population in this area. It is known for Scolioidea, where Tiphia belongs, that adults preferably feed on nectar and pollen of umbelliferes, apparently for survival and as a protein source for egg production. Pots with cut flowers of umbelliferes, wild carrot (Daucus carota), hogweed (Heracleum sphondylum) and upright hedge-parsley (Torilis japonica) were placed in the experimental area of the golfcourse. No naturally occurring umbellifers were found on the golfcourse. Around the study area wild flowers were checked for adults of T. femorata. Exclusively the flowers of umbellifers appeared to be highly attractive for the parasite. It might very well be possible to promote these parasites in planting wild carrot in and around the edges of the field.

In western Europe two tipulid species play an important role in grassland. T. paludosa Meigen and T. oleracea Linnaeus are wide-spread, T. paludosa has also been recorded from North America as a pest (South-Western British Columbia and North-Western Oregon). Damage has been reported on beet-seedlings, winter wheat, various vegetable crops and grasses (pastures, sportfields and amenity grass). T. paludosa is considered to be the most abundant whereas T. oleracea, T. subcunctans (is T. czizeki), Lunatipula vernalis Meigen and Nephrotoma maculata generally play a role of minor importance. T. subcunctans has been reported from the North Western part of Germany where it can be abundant in wet pastures T. paludosa is an univoltine species, which lays its eggs from late August until the beginning of October. Larval stages are found throughout October to June or July the next year; it hibernates in the 2nd or 3rd instar without diapause. T. oleracea is a bivoltine species with a flight period in May and again in late July and August. Larvae of this species overwinter the same way as the former one. The univoltine species T. subcunctans has its flight period in October and eggs overwinter. T. paludosa

and T. oleracea have been found in various (normally rather wet) habitats. T. paludosa has frequently been found in dry sandy soil as well.

It is known for Tipulidae that the population shows a periodicity of about seven years. It is suggested that the decline of the population could be caused by Tipulid Iridescence Virus (TIV). Periodicity is a common phenomenon in insect populations, but in all investigated cases there has not been found a particular cause. We found that population crashes can be caused by frost periods without snow-cover.
L1 larvae feed on the young leaves of grasses. Older instars feed at the base of the stem. Young grass plants with two or three leaves can be cut off by older instars and are pulled down into their holes where they are eaten (Vlug, 1990). Generally it was accepted that leatherjackets feed on the roots of grasses but we proved that leatherjackets cannot survive on roots, but need green leaf tissue (Vlug & Harrewijn, 1994). Under certain conditions leatherjackets could be reared in soil with a high amount of dead organic matter without living plant material.

Possibilities for biological control

Pastures:

Positive results have been obtained with BTI against leatherjackets in early winter. Detailed studies on the role of BTI in grassland against laetherjackets are in preparation. The effect of BTI against bibionid larvae has not been studied, but might very well be possible. Fritflies are commonly parasitized by natural occurring hymenopterous parasites, in some cases it remains a problem. In Italy and Switzerland white grubs of may- beetles are controlled by the fungus disease Beauveria brognarti. The garden chafer populations can be influenced by planting wild carrot to promote its parasite Tiphia femorata.

Sportfields:

The use of parasitic nematodes against white grubs of garden chafer seems promising if circumstances are optimal. The parasite Tiphia femorata could play a role of importance. Other grub species are less or not affected by nematodes and hymenopterous parasites.

Control of grubs of may-beetles by Beauveria has not been carried out in The Netherlands as yet, but might be possible. In bioassays the performance of the nematode Steinernema glaseri on may-beetle improved after trough-rearings of the nematod on the grubs (Tab. 1). Noctuid larvae could be controlled by nematodes. The use of Acremonium (endophytes) is restricted but not studied yet.

- Since Tipulid larvae are known feeding on grass leaves, endophytes may play a role in control of them.
- Frit fly larvae as stem borers are not affected by endophytes.
- Bibionid larvae feed on the growing point of the grass or just beneath it on the root system. They do not feed below soil surface. Influence of endophytes is unknown.
- Scarabeid larvae are typical root feeders and not affected by endophytes(?)
- Noctuid larvae are typical root feeders and not affected by endophytes(?)

Tab. 1. Performance of S. glaseri in bioassays against grubs of M. melolontha.

	living	dead	%dead
blanco	29	1	3.3
S. glaseri #326	9	21	67.7
S. glaseri S-1	3	27	90.0
S. glaseri S-2	0	30	100.0

Literature

Vlug, H.J., 1990. Feeding behaviour of Tipulid larvae on grass. Med. Fac. Landbouww. Rijksuniv. Gent, 55:545-547.

Vlug, H.J. and Harrewijn, P., 1994. Analysis of gut contents and feeding behavior of tipulid larvae (Diptera: Tipulidae) using a new root-staining technique. J. Econ. Entomol., 87:101-102.

Observations on Canadian grasses

Werner Gieffers and Sabine Spiegelhauer-Könn,

Max-Planck-Institut für Züchtungsforschung Köln, GERMANY

Abstract

The grass species *Agropyron dasystachium, A. subsecundum, A. riparium, A. trachycaulum, Festuca ovina* var. *canadensis , Deschampsia caespitosa, Poa alpina, P. interior, P. stenatha* and *Trisetum spicatum* , which come from a breeder in Canada were tested in a field trial in Cologne. The field trial experiment was designed in order to analyse allelopathic reactions against weeds. Furthermore the infection of these grass species by fungi was determined as well as the spectrum of fungal pathogens. The following results are based on investigations over one year.

A. trachycaulum, F. ovina var. *canadensis, and Trisetum spicatum* decreased the growth of dicotyledonous weed clearly. These grasses in order of species decreased weed infestation by 88.8, 76.3, and 68.4 per cent. This appears to be an allelopathic reaction.

Infection by fungal pathogens was ascertained in the field by estimating the chlorotic and necrotic symptoms. The same three species *A. trachycaulum, F. ovina* var. *canadensis,* and *T. spicatum* also showed a low degree of infection. The correlation between reduced weed infestation and low infection was significantly.

The identification of fungal pathogens was made by microscopically analysis of spores. For this the infected leaf material was incubated under special sporulation conditions.

A number of 16 fungal pathogens could be identified and is listed in order of frequency: *Alternaria alternata, A.* spp., *Ulocladium spp., Epicoccum nigrum, Fusarium spp., Drechslera spp., Pseudocercosporella herpotrichoides, Pleospora infectoria, Erysiphe graminis, Puccinia spp., Gelasinospora cerealis, Torula graminis, Stemphylium* spp., *Septoria nodorum, Spermospora subulata, and Botrytis cinerera.*

Introduction

Weeds in grasses are controlled usually by tillage methods and by use of herbicides. Like other plants, weeds have a lot of pathogens and enemies. Biological control sometimes is possible by infection with such pathogens. It seems also to be possible by using grasses with allelopathy to control weed infestation. This form of biological control is to our knowlegde quite unknown. In most cases allelopathic reactions are low and their efficacy to reduce weed growth is insufficient.

In the last years WEIJER (1993, unpublished) in Canada found in the genera *Agropyron* and *Festuca* a few grass species with allelopathy against dicot weeds.

Material and methods

In co-operation with the company Lauff in Cologne we designed a field trial in the breeding field of the Max-Planck-Institute in Cologne to investigate allelopathic reactions in different grass species, which we obtained from Canada.
Two years, from 1991 to 1992, the trial area was without cultures. On this fallow a weed population of high density was grown. After them from 1993 until the beginning of the field experiment white clover was grown.
The field trial started in June 1994. Twelve grass species were sown in plots of ten square meter. Four species of *Agropyron* were cultivated in six, the other grasses in three repetitions.
Table 1 shows the grasses used in the field trial. WEIJER (1993, unpublished) found in this three *Agropyron* species allelopathy reactions against dicot weeds and also in *Festuca ovina* var. *canadensis*. In the roots of these grasses he was able to detect two compounds but he did not reveal us the chemical composition. Allelopathy in the other grasses is supposed by him but not proved.

Furthermore the infection of these grass species diseased by fungi in the field was estimated in per cent by using the so-called „Komplexbonitur" (GIEFFERS et al. 1988). This estimation covers altogether the symptoms caused by all fungal pathogens.

Table 1 Grass species used

Area of distribution			
North America		Africa, Asia, Europe, North-America	
Species	Efficacy of allelopathy	Species	Efficacy of allelopathy
Agropyron		*Deschampsia*	
A. riparium	proved	*D. caespitosa*	unknown
A. trachycaulum	proved		
A. subsecundum	proved		
A. dasystachium	unknown		
Festuca		*Poa*	
F. ovina var. *canadensis*	proved	*P. alpina*	unknown
Poa		*Trisetum*	
P. interior	unknown	*T. spicatum*	unknown
P. stenatha	unknown		

In addition the spectrum of fungal pathogens were identified. Leaf peaces cut in 3 cm length, with disease symptoms were incubated under the conditions 10 and 17° C, permanent watering, UV-radiation and hydrogen peroxide (GIEFFERS et al. 1989, FLADUNG et al. 1993) After an incubation time of 10 to 14 days the leaves were examined by microscopically analysis.

Results and discussion

Weeds

After sowing the weed growth was equal on all plots. In August two month after sowing we examined the weed flora of the plots for the first time. The weeds of all plots richer a cover percentage from 90 to 100 per cent (Fig. 1).

In 1994 we found five weeds with high frequency (more than 50 plants per plot /10 m^2): *Chenopodium album, Galinsoga ciliata, Matricaria chamomilla, Sinapis arvense, Stellaria media* and *Veronica arvensis*.

In the vegetation period in 1995, we observed in June the highest weed infestation (Fig. 1). In this time we found the same weeds, except *Galinsoga ciliata* but in addition three other weeds (*Capsella-bursa-pastoris, Plantago artrata* and *Sonchus olera-ceaeus*), however the frequency was smaller.

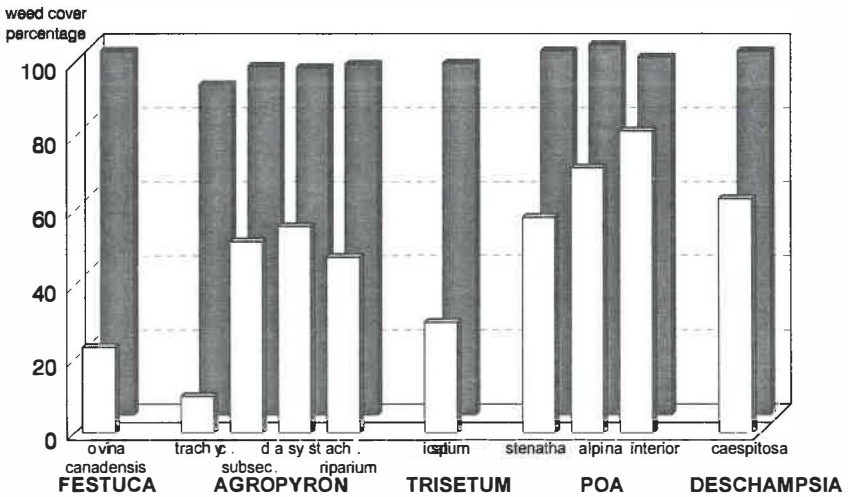


Fig.1 Weed infestation on several grass species,
August 1994 black, June 1995 white

The efficacy of allelopathy depends from the plant stage. Therefore two month after sowing is a short time and a weed reduction was not expected.

However eleven month later in June 1995 (Fig. 1) another situation was established. The results in June 1995 showed, that the intensity of weed infestation was very small by *F. ovina* var. *canadensi, T. spicatum* and especially by *A. trachycaulum.*

We found in plots from *A. trachycaulum* two or three plants only from *Epilobium* or *Sonchus*. The green plot area of this grass was surrounded only from weeds of other plots or from the path.

However *Plantago atrata* occurred with higher frequency but in July their leaves were chlorotic and the plants decayed.

The last analysis of weed cover percentage in the vegetation period 1995 was made in September (Fig. 2). The weed cover was decreased in all grasses, but the same species showed the lowest weed infestation. In both analysis *F. ovina* var. *canadensis, T.*

spicatum and especially *A. trachycaulum* demonstrated the lowest weed cover. The weed cover percentage of these three grasses are significantly lower as the data of the other grasses.

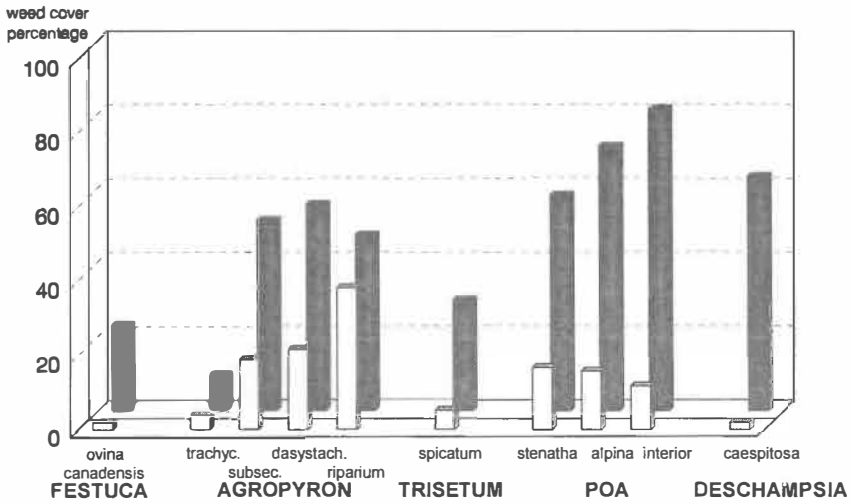


Fig. 2 Weed infestation on several grass species 1995
June black and September white

The data of *D. caespitosa* showed a high difference. It is known, that allelopathic reactions can start after one, two or three years, when the root is completely deve-loped. Perhaps it is possible to find this low infestation in the next years again.
It seems to be possible, that the reduction of weed infestation observed in these three grasses is caused by allelopathy.

Fungal pathogens

In addition to the weed analysis we estimated the leaf infection and detected also the fungal pathogens. The chlorotic and necrotic symptoms of the plant in the plot are estimated in per cent. Two questions led us to perform this infection and pathogen analysis:

- Are there a correlation between the reduction of the weed infestation and of the diseases?

- Which infection degree show Canadian grasses under European conditions and which pathogens infect these grasses?

In the field trial the infection analysis started in September 1994 three month after sowing. Four grasses reacted with high infection (Fig. 3). The other species were significantly low infected. A further disease estimation in May 1995 demonstrated similar results.

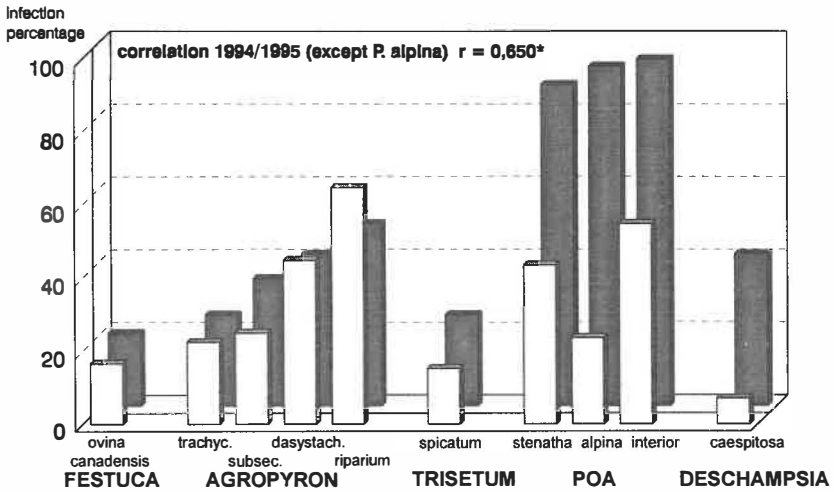


Fig. 3 Leaf infection on several grass species
September 1994 black and September 1995 white

One year later the analysis from September 1995 showed, that three grasses only reproduced the low infection level from 1994 (Fig. 3). Between the first and the last infection analysis the disease infestation correlated in most cases.

The infection data from September 1994 correlated significantly to the weed infestation from September 1995 (Fig. 2) with r = 0.802 and the infection from September 1995 also correlated significantly to the weed infestation from June 1995 with r = 0,812.

In our investigations *A. trachycaulum, F. ovina* var. *canadensis, and T. spicatum* confirm the expected reduction of weed. They decreased the growth of dicotyledonous weed clearly. In addition these three grasses demonstrated a high resistance to fungal pathogens, too. Further investigations are necessary to clarify relations between

allelopathy and the fungal resistance found and to reproduce this first results from one year.

In another study we detected fungal pathogens on infected grass leaves. During the month July to September altogether 16 pathogens were identified. The data are listed in Table 2.

Table 2 Host plants for 16 fungal pathogens found

Host	*Deschampsia*	*Agropyron*				*Festuca*	*Trisetum*	*Poa*			Host Number
Pathogen	*caespitisa*	*trachycaulum*	*riparium*	*subsecundum*	*dasystachium*	*ovina var. canad.*	*spicatum*	*alpina*	*interior*	*stenatha*	
*Alternaria alternata, A.*spp	+	+	+	+	+	+	+	+	+	+	10
Ulocladium spp.	+	+	+	+	+	+	+	+	+	+	10
Epicoccum nigrum	+	+	+	+	+	+	+	+	+		9
Fusarium spp.	+	+	+	+	+	+		+	+	+	9
Drechslera spp.	+	+	+	+	+	+	+			+	8
Pseudocercosporella herporichoides	+	+	+			+		+	+	+	7
Pleospora infectoria	+			+		+	+	+	+	+	7
Erysiphe graminis	+		+	+		+	+		+		6
Puccinia spp.	+	+	+		+		+	+			6
Gelasinospora cerealis	+	+	+				+	+			5
Torula graminis	+			+			+	+		+	5
Stemphylium spp.			+		+				+		3
Septoria nodorum	+	+				+					3
Spermospora subulata		+								+	2
Botrytis cinerea	+										1
Pathogen Number	13	10	10	8	7	9	9	9	8	8	

Infection reactions on Canadian and European grasses were similar. The data from Table 2 compared to the other data gave no correlation neither to the infection level nor to the weed cover percentage.

Field trials with allelopathic grasses about weed reduction and their correlation to infection by fungi will be continued. Further investigations are necessary to study this phenomena in more details.

References

Gieffers, W. und j. Hesselbach, 1988. Krankheitsbefall und Ertrag verschiedener Getreidesorten IM Rein- undo Mischanbau. I. Sommergerste (*Hordeum vulgare* L.).
J. Plant Diseases and Protection 95, 1, 46-62.

Gieffers, W., V.H. Paul und E. Ritter, 1989. Der Einfluß von Sauerstoff und UV-Licht auf die Konidienproduktion von *Pseudocercosporella herpotrichoides* (Fron) Deighton, Merkmale zur Morphologie des Erregers und dessen Nachweis an Dikotyledonen.
J. Phytopathologie 126, 115-132.

Fladung, M. and W. Gieffers, 1993. Resistance reactions of leaves and tubers of *rolC* transgenic tetraploid potato to bacterial and fungal pathogens. Correlation with sugar, starch and chlorophyll content. Physiological and Molecular Plant Physiology 42, 123-132.

Histological effects of ozone on the tissues of leaves of Lolium perenne

Masuch, G., Franz, J.-Th., Schoene, K., Wiese, P.

University of Paderborn

Introduction

In clean-air areas of the Egge Mountains, far away from emission sources, forest trees and herbs are generally exposed to mixtures of gaseous pollutants. The main component of air pollutants is ozone, whereas there are background concentrations of nitrogen dioxide and sulfur dioxide.

The measuring station of the LIS (Landesumweltamt) in the Egge Mountains reveals detailed information about immission patterns.

Open-Top-Chambers are well suited to elucidate the effects of immissions in natural stands comparing plants grown in chambers with ambient air with those grown in chambers aerated with charcoal-filtered air.

Materials and Methods

Plants of Perennial ryegrass (Lolium perenne cv. Limes) were cultivated in Open-Top-Chambers in a forest site of the Egge Mountains in Northrhine-Westphalia, 440 m above sea level, from May to October in 1993. This experiment was repeated in 1994.One experimental series in both years was aerated with ambient air containing high ozone immission loads, low sulfur dioxide and low nitrogen dioxide concentrations. The other experimental series was aerated with charcoal-filtered air.

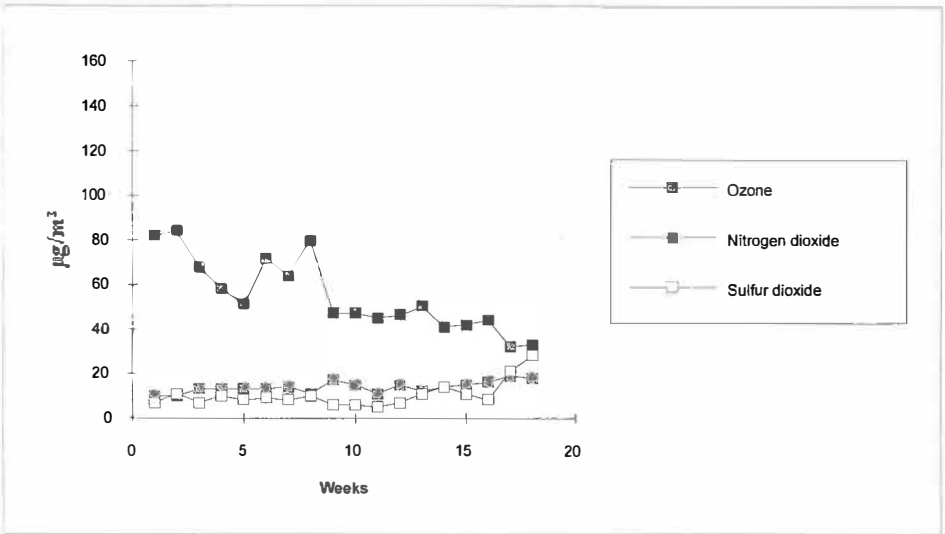
Leaf tissues of the second youngest leaf, which was fully developed, were compared quantitatively.

Samples of the leaf blades were prepared by fixation in 2.5% glutardialdehyde in 0.2 M phosphate buffer for 1 h. The samples were dehydrated in an ethanol series and embedded in styrene methacrylate. Semi-thin sections were cut on a LKB Ultrotome III, stained with

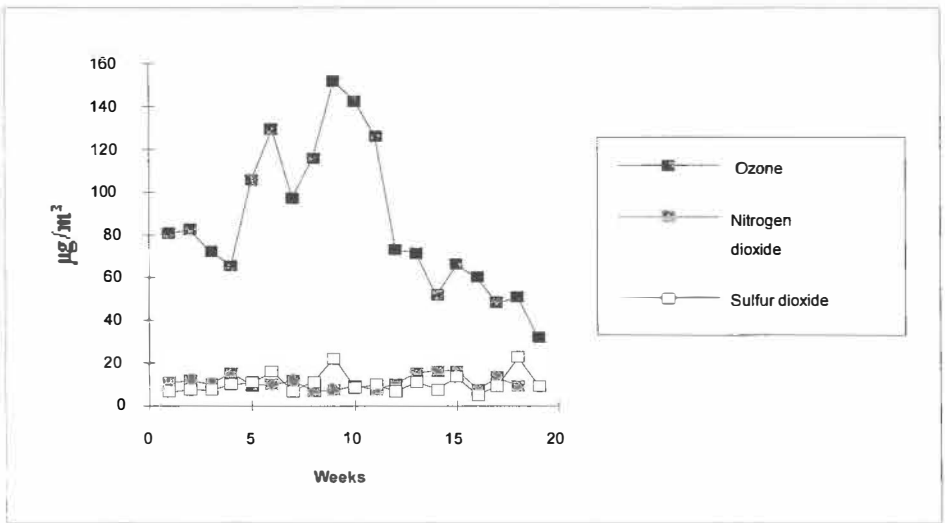
toluidine blue, photographed with a Leitz Orthomat and analysed quantitatively with a graphic tablet connected to a PC.
Amyl-acetate was used as a solvent to extract resin from semi-thin sections for SEM observation. Electron microscopic studies were performed with a Hitachi H 3010 scanning electron microscope.

Results and Discussion

Leaf tissues, exposed to high ozone concentrations, showed some typical alterations. The parenchymatous bundle sheath is slightly dilatated. Xylem tissues and especially xylem vessels (tracheas) are enlarged. On the other hand the phloem tissues are reduced. These phenomena point to features of water stress. Due to a reduced transport system the assimilates are prevented from being removed from the site of production. Structural changes of the chloroplasts can be observed in mesophyll cells of plants exposed to ambient air containing high ozone concentration and background concentrations of nitrogen dioxide and sulfur dioxide; their chloroplasts are enlarged and show dilatations of the thylakoidal membranes. Chloroplasts of reference leaves, exposed to charcoal-filtered air, are filled up with thylakoids without dilatations between them.



Weekly mean immission values in the ambient air of the Egge Mountains from 25.06.-01.10.1993



Weekly mean immission values in the ambient air of the Egge Mountains from 25.05.-04.10.1994