Forage Pathology and Endophytes in Tropical Grasses

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I. Anthracnose Disease of *Stylosanthes*

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., is one of the major limitations to the extensive use of *Stylosanthes* as a tropical forage legume. Dry matter losses of up to 100% have been reported for *S. guianensis* in Colombia and elsewhere. Disease symptoms on *S. guianensis* include necrosis and blight on above ground plant parts. The pathogen exhibits extensive variation in morphology and pathogenicity. Two separate biotypes of the pathogen, designated A and B, have been reported as causing symptoms on species of *Stylosanthes* in Australia.

C. gloeosporioides is one of the most important pathogens infecting a wide range of crops.

Summary of Major Research Findings

- We tested and assembled an international set of *S. guianensis* differentials which are successfully being used to differentiate

isolates of *C. gloeosporioides* into races (Kelemu et al.1996. Plant Disease 80:1355-1358).

- We have done several years of genetic analysis work on the pathogen population. We concluded that the South American pathogen population is highly diverse and complex with many intriguing features (Badel and Kelemu.1997. Pasturas Tropicales 19:2-; Kelemu, et al.1997. Tropical Grasslands 31:387; Kelemu, et al.1999. European Journal of Plant Pathology 105:261-; Kelemu,et al. 2000. Pasturas Tropicales 22:16-; Kelemu et al.1995. Phytopathology 85:1201; Kelemu, et al. 1999. Phytopathology 89: S38).
- A confusing and troublesome new disease of *Stylosanthes* which complicated anthracnose evaluations has been conclusively identified and characterized (Kelemu et al. 1997. Canadian Journal of Plant Pathology 19: 376-).
- A transposon element has been identified, cloned and characterized in the anthracnose pathogen, as part of a PhD thesis, in a collaborative project with Dr. John Manners, University of Queensland/CSIRO and myself (He et al. 1996. Mol. Gen Genet 252:320-).
- The distribution and relationship of chromosome-specific dispensable DNA sequences have been studied in diverse isolates of *C. gloeosporioides*, as part of a PhD thesis, in a collaborative project with Dr. John Manners, University of Queensland/CSIRO, and myself (He et al. 1995. Mycol. Res. 99:1325).
- Several lines and accessions of *Stylosanthes* have been identified with high levels of anthracnose resistance.
- We have conducted work on other methods of anthracnose disease management (Kelemu and Badel. 1994. Australasian Plant Pathology 23:41; Jiang and Kelemu. 1999. MPMI abstract).
- Field epidemiology work to study conditions for anthracnose disease development has been completed (Chakraboty et al. 1997. Tropical Grasslands 31: 408)

Funding

AIDAB (1992-1994), ACIAR (1995-2001), Australia

Capacity Building/Collaboration

- A Chinese PhD student in collaboration with University of Queensland (1993-1996); currently Associate professor at a university in Beijing.
- One Chinese MSc student; currently a PhD student at a university in China.
- One Brazilian visiting scientist; currently a PhD student at a university in Brazil
- One Colombian undergraduate student (female); currently a PhD student in Chile
- One Chinese visiting scientist 2000/2001
- Several Colombian research assistants who have worked in the lab on various aspects of *Colletotrichum* are PhD students abroad (one at Cornell University; one at Univ. of Florida; one (female) at University of Bath, England)
- Several short term visitors and trainees (ranging from one week to 3 months)
- University of Queensland, CSIRO, EMBRAPA, CATAS (China), Kansas State University/USDA, IGFRI (India), CORPOICA/ICA.

II. *Rhizoctonia* Foliar Blight of *Brachiaria*

Rhizoctonia foliar blight is of increasing importance not only in *Brachiaria*, but also with other crops (eg. rice). Generally speaking there are no good sources of resistance in many crops because of the lack of specialization in the pathogen and the general primitive nature of the pathogen. In addition, control strategies are difficult as the pathogen can survive in the soil as hardy sclerotia for several years. These sclerotia form on plant tissues and shed like seeds as they mature.

Summary of Major Research Findings

- We have developed an effective inoculation method both in the field and in the greenhouse (Kelemu et al. 1995. Tropical Grasslands 29:257-262).
- One hundred eighty *Brachiaria* hybrids screened for resistance to *Rhizoctonia* foliar blight in year 2001. Criteria used to evaluate: percentage of infected leaves and upward disease progress from the inoculation point

- Of these, 14 were rated as resistant (up to 5-cm upward disease progress from the inoculation point and up to 10% infected leaves)
- Screening for sources of resistance will continue in 2002 and beyond
- Work on conditions for production and germination of sclerotia (for disease management strategies) will be conducted.

III. Bacterial Blight/Wilt of *Brachiaria*

Xanthomonas campestris pv. *graminis* has been identified as the causal agent of bacterial wilt of species of *Brachiaria*. Commonly occurring symptoms in *Brachiaria* are curling and eventual wilting of leaves as well as chlorotic stripes. Entire plant death may result in very susceptible plants under favorable conditions.

Summary of Major Research Findings

- Some isolates of *X. campestris* pv. *graminis* have been collected from different *Brachiaria* genotypes for further characterizations.
- Effective artificial inoculation methods have been developed.
- Sources of resistance have been identified in species of *Brachiaria* (Zuleta et al. 2002. Manejo Integrado de Plagas (in press).
- Isolate collections and characterizations will continue.

IV. Disease Management Strategies

Host resistance is the most important, environmentally safe method for controlling plant diseases. Plants have highly efficient defense mechanisms to fend off attacks by microbes. We focus mainly on strategies to understand and enhance host resistance. We study the race compositions, the geographic distribution of the various races of pathogens and the genetic diversity of pathogen isolates in order to help develop effective breeding programs for disease resistance and of gene deployment strategies for managing the resistance.

For diseases with no known host resistance, we study alternative disease management strategies. For example, we introduced a rice-chitinase gene into the widely grown *S. guianensis* accession CIAT 184 to enhance foliar blight (caused by *Rhizoctonia solani*) resistance

(Kelemu et al. 2001. Phytopathology 91:S47). Chitinases have been reported from several plants and various microbes. In plants, both biotic and abiotic stresses can induce chitinase expression at relatively high levels, although, constitutionally, many plant chitinases are often expressed at low levels. Chitinase catalyzes the hydrolysis of the β-1,4 linkages of the N-acetyl-D-glucosamine polymer chitin, a structural component in many organisms, but absent in plants. In contrast, chitin constitutes between 3% and 60% of cell walls in fungi.

We have started studies on biocidal proteins isolates from tropical forage legumes. We think that these plant proteins could play an important role in biotechnological approaches to enhance host plant resistance to plant pathogens. We have isolated, purified and characterized a small peptide from a forage legume which has potent biocidal properties.

V. Endophyte/*Brachiaria* Symbiosis

Endophytic fungi form nonpathogenic and intercellular associations with grasses, completing their life cycle within the plants' aerial portions. Many grasses harbor endophytic fungi, which are among the most widely used biological plant protection agents in forage and turf grasses. Endophyte containing plants also possess a number of other properties of applied value such as drought tolerance, vigor, and persistence.

It has been demonstrated that the greatest impact of endophyte-infected temperate grasses is to reduce livestock productivity. It remains to be seen whether this is true for tropical forage grasses such as species of *Brachiaria*.

To the best of our knowledge, CIAT is the only CGIAR Center working on tropical grass/endophyte associations.

Summary of Major Research Findings

- We have shown that various endophytic fungi inhabit native savanna grasses (Koga et al. 1995. Plant Disease 79: 1074) and introduced forage grasses such as *Brachiaria* (Kelemu and Takayama, 1998. Phytopathology 88:S46; Kelemu et al. 2001. Canadian Journal of Microbiology 47:55-62)

- To date, we have 11 isolates of endophytes from species of *Brachiaria.*
- We have determined conclusively how related each of the 11 isolates are to each other and to other endophytes from other plants.
- We have developed inoculation methods and we can now artificially introduce endophytes in various genotypes of *Brachiaria* (more success in some than in others).
- We have determined that these endophytes play a role in the protection of *Brachiaria* from some fungal diseases (eg. leaf spot disease caused by *Drechslera* spp.) and some insects (eg. aphids).
- We have cloned and sequenced a DNA fragment common to most of the endophyte isolates. Based on this sequence data, we designed and synthesized specific primers to be used for a PCR-based detection method. This method is highly sensitive, fast and reliable in determining the presence or absence of endophytes in *Brachiaria*. It can detect all of the 11 isolates we have to date. Pathogenic and non-pathogenic fungi other than endophytes cannot be detected with these primers. This work and these results have greatly facilitated our efforts in screening and determining the presence or absence of endophytes in various parts of the plant.
- We have determined conditions to completely eliminate endophytes from *Brachiaria*, thus generating genetically identical clones with and without endophytes.
- Results from greenhouse conducted experiments indicate that endophytes may have a role in drought tolerance in *Brachiaria*.
- Preliminary results indicate that resistance to *Rhizoctonia* foliar blight in *Brachiaria* may be due to the presence of endophytes.

Funding

We have been receiving funds from the Government of Japan to support this work since1995.

Capacity Building/Collaboration

- One Japanese young scientist (female, JOCV/JICA) 1998-2001
- One Chinese visiting scientist 2000-2001 (1.5 years)
- One Japanese young scientist (female, JOCV/JICA) 2002- 2004

- Two senior Japanese visiting scientists (collaborators) 1998, 1999, 2000
- Two undergraduate Colombian students (females) as trainees for 3 months 1999
- One Brazilian (female) visiting scientist, currently a PhD student in Brazil
- One Chinese PhD student (2001-)
- Rutgers University, NJ, USA (molecular taxonomy)

Future Work

- Alkaloid determination (collaborative)
- Animal toxicity studies (depends on full funding)
- Confirmation of disease resistance (*Rhizoctonia*)
- Drought tolerance studies in the field
- Disease resistance studies in the field
- Studies on *Brachiaria*/endophyte interactions and cell associations (collaborative)

S. Kelemu Profile

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EDUCATION

1974 - 1979	B.Sc. Addis Ababa University, Ethiopia. (Honors), Plant Science.
1983 - 1984	M.Sc. Montana State University, Bozeman, Montana, USA Plant Pathology/Genetics.
1985 - 1989	Ph.D. Kansas State University, Manhattan, Kansas, USA Plant Pathology/Molecular Biology
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PROFESSIONAL EXPERIENCE

1992- present	Senior Scientist, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia
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1985-1989	Ph.D. Graduate Research Assistant, Dept. of Plant Pathology, Kansas State University, Manhattan, KS. 66506, USA
1983-1984	M.Sc. Graduate Research Assistant, Dept. of Plant Pathology, Montana State University, Bozeman, MT. 59717, USA
1982	Lecturer and Researcher, Dept. of Plant Pathology, Addis Ababa University, Ethiopia
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1979-1981	Assistant Lecturer and Researcher, Dept. of Crop Protection, Addis Ababa University, Ethiopia

PUBLICATIONS (SELECTED)

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