APPENDIX 2

PUBLICATIONS AND OTHER DISSEMINATION

Published:
- CHIPILI, J., SREENIVASAPRASAD, S., TALBOT, N. J. and SERE, Y.(1998) Genetic diversity of the rice blast pathogen populations in West Africa. Second International Rice Blast Conference, Montpellier, France. 4 - 8 August, 1998. Poster. Abstract included in Conference Proceedings. (B) [SEE APPENDIX 5]
- CHIPLILI, J., SREENIVASAPRASAD, S. and TALBOT, N. J. (1999). Genotype and pathotype diversity of the rice blast pathogen *Magnaporthe grisea* in West Africa. Offered presentation at the Molecular Biology of Fungal Pathogens X Conference, University College of North Wales, 7 - 9 July 1999. (B)
- CHIPILI, J. (2000) PhD thesis on 'Characterisation of populations of *Magnaporthe grisea*, the rice blast fungus, in some of the West African countries'. (E)
- SREENIVASAPRASAD, S., CHIPILI, J. and SERE, Y.(1998) Molecular characterisation of the blast pathogen *Magnaporthe grisea* from some West African rice screening sites. International Congress of Plant Pathology 1998, Edinburgh, U.K. 9 - 16 August 1998. Poster. Abstract included in Conference Proceedings. (B) [SEE APPENDIX 6]
- SREENIVASAPRASAD, S. (2000). Isolation of Fungal Nucleic Acids. *In: Nucleic Acids Protocols Handbook*. pp 37-45. English. (Eds.) Rapley, R. and Walker, J. M. Humana Press, U.S.A. (A)
- TURNER, H. C. (1998) Molecular variability of the rice leaf scald pathogen *Monographella albescens*. International Congress of Plant Pathology 1998, Edinburgh, U.K. 9 - 16 August 1998. Poster. Abstract included in Conference Proceedings. (B) [SEE APPENDIX 4]

In press:
- CHIPILI, J., SREENIVASAPRASAD, S., SERE, Y. and TALBOT, N.J. (2000). Characterisation of the rice blast pathogen population in west Africa. In: *Major Fungal Diseases of Rice Current Status and Perspectives*. English. (Eds.) Sreenivasaprasad, S. and Johnson, R. Publisher: Kluwer Academic Publishers, Dordrecht, the Netherlands (A).
- TURNER, H. C. & BLACK, R. (2000) Rice leaf scald: pathogen biology and diversity. In: *Major Fungal Diseases of Rice*. English. (Eds.) Sreenivasaprasad, S. & Johnson, R. Publisher: Kluwer Academic Publishers, Dordrecht, the Netherlands (A).

In preparation:
- TURNER H. C., SERE Y., TWUMASI J., NUTSUGAH S. K., BROWN A. E. and BLACK R. Molecular variability in W. African populations of the rice leaf scald pathogen, *Monographella albescens*. (A)
- TURNER, H. C., SREENIVASAPRASAD, S. and HOLDERNESS, M. (1998). Invited presentation of project activities and outputs at the CPP workshop on Rice Crop Protection in Africa, Natural Resources Institute, Natural Resources Institute, Chatham, U.K. 7 - 8 December 1998. (A)
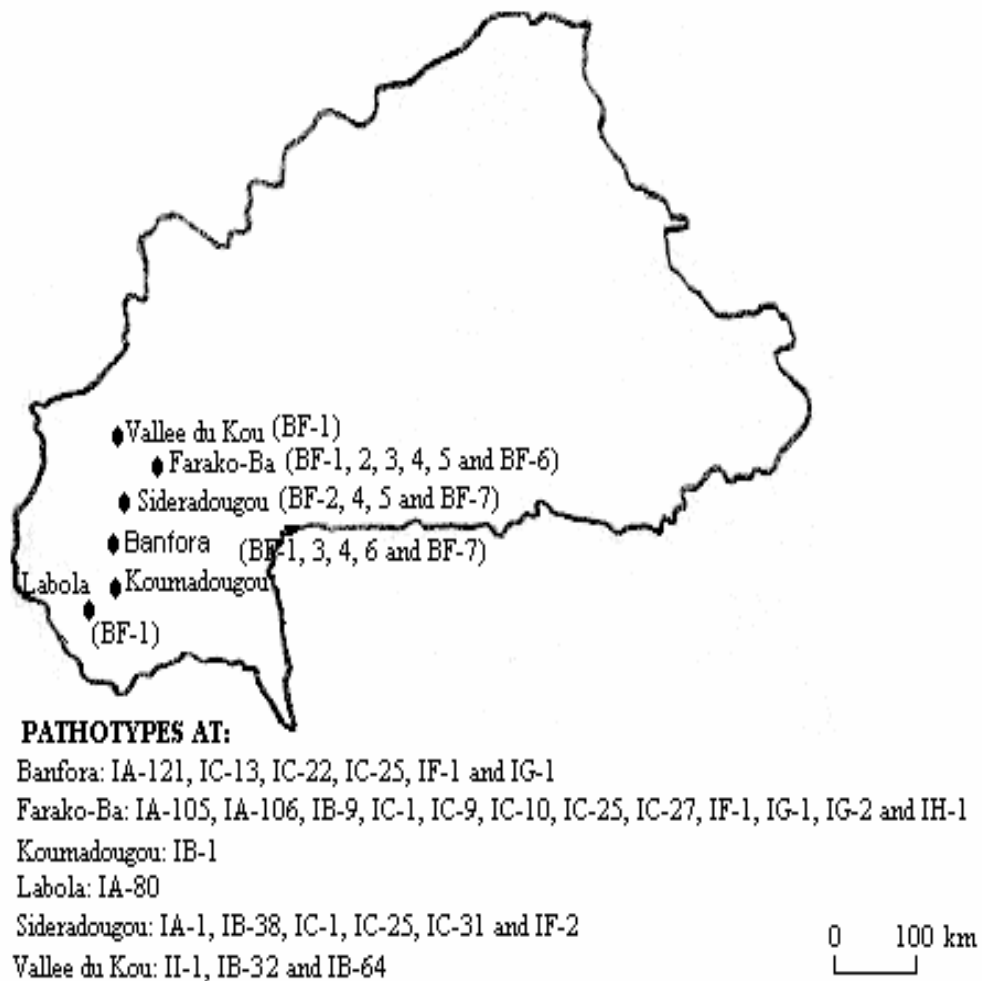
Internal reports:
All quarterly and annual reports submitted to DfID deadlines.
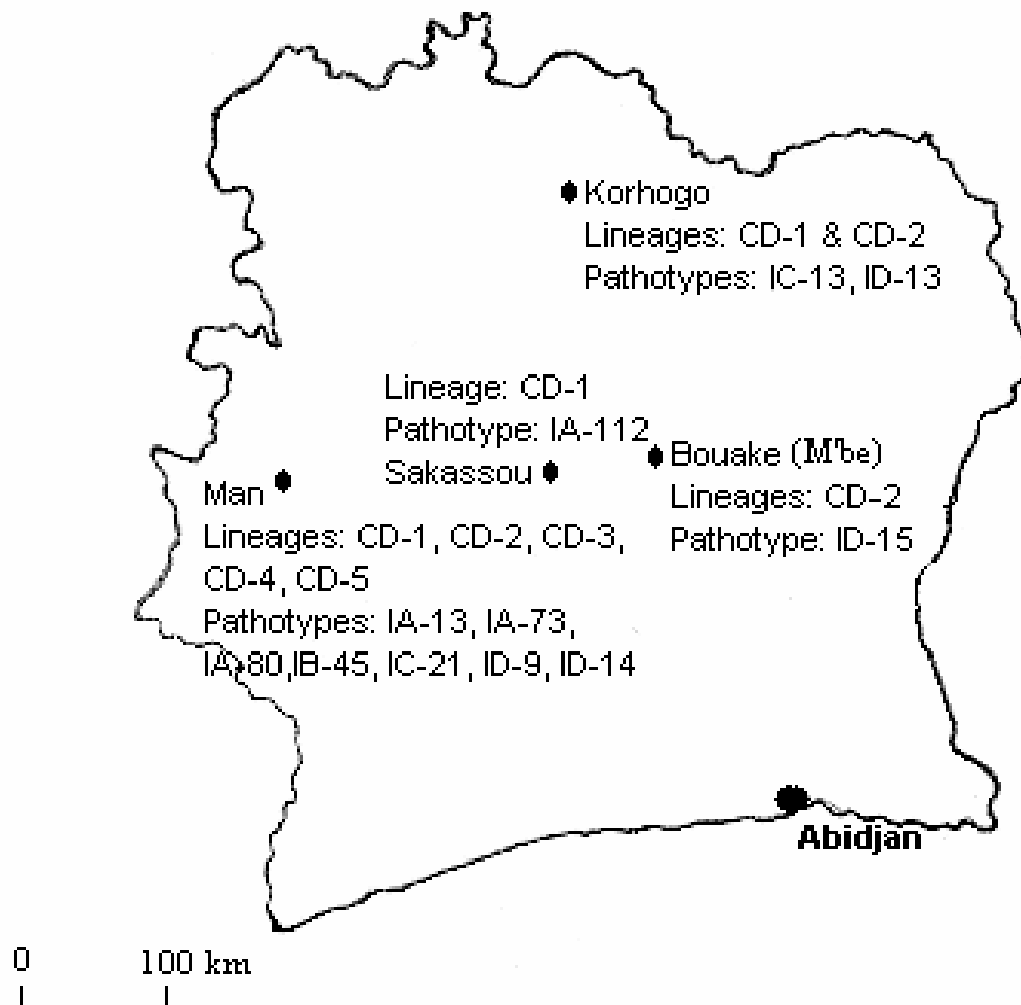Back-to-Office Reports as follows:
- BANNIZA, S. (1997) Report on survey visit to the Crops Research Institute and the Savannah Agricultural Research Institute, Ghana. 5 September - 19 September, 1997. Project R6738. Egham, CABI Bioscience. pp. 6. (C)
- BANNIZA, S. (1997) Report on Liaison visit to the West African Rice Development Association (WARDA), Côte d' Ivoire; the Crops Research Institute, Ghana; and the Savanna Agricultural Research Institute, Ghana. 11 March - 24 March, 1997. Project R6738. Egham, CABI Bioscience. pp. 4. (C)
- RUTHERFORD, M. (1998) Report on a visit to Ghana to undertake survey and sample collection of rice blast and scald. 23 October -31 October, 1998. Project R6738. Egham, CABI Bioscience. pp. 5. (C)
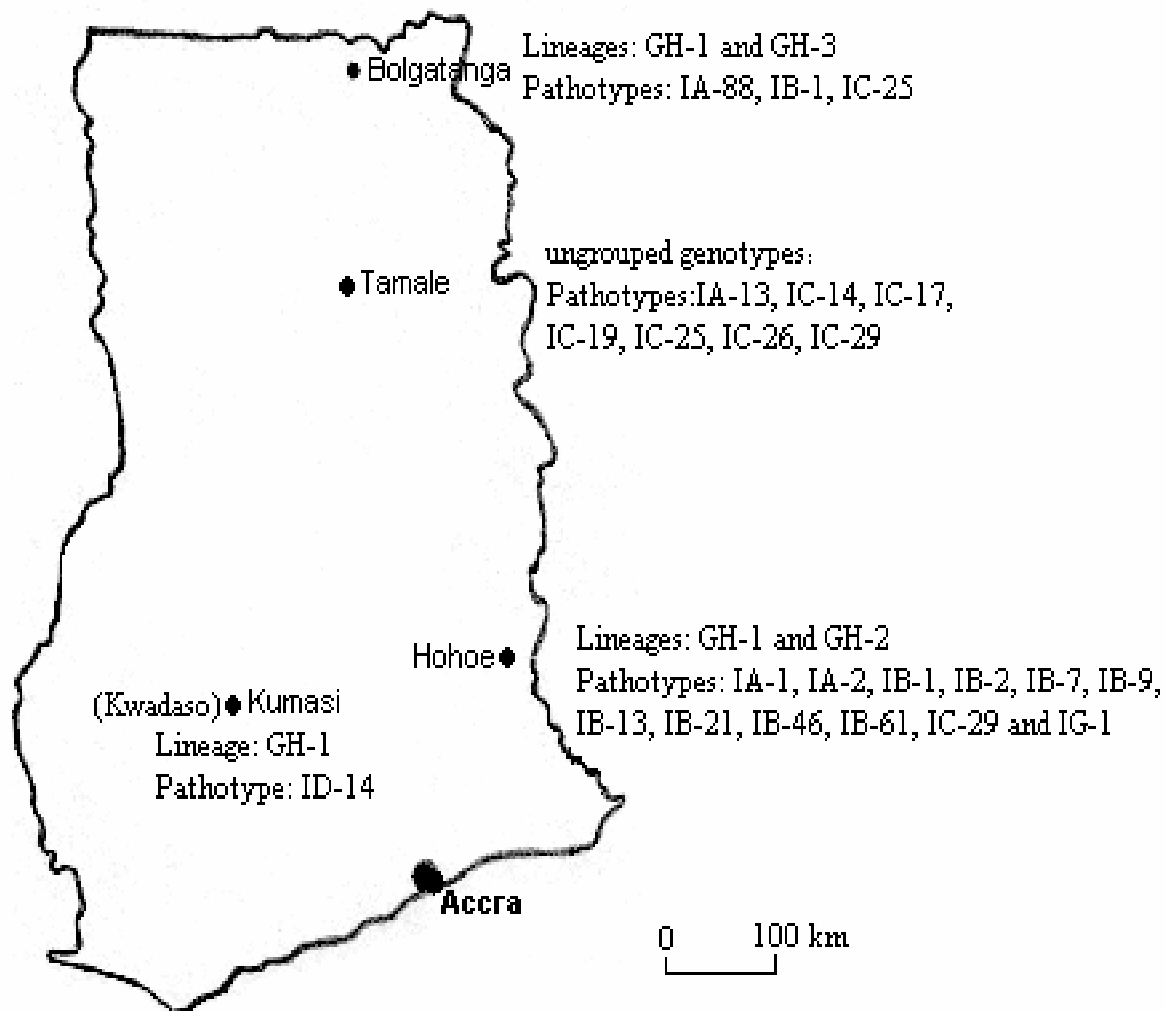
Other dissemination of results, training etc.:

- CHIPILI, J. (1997) First year Ph.D. report (Oct. 1996 - Oct. 1997). Submitted to the University of Exeter.(G)
- CHIPILI, J. (1998) Characterisation of the rice blast pathogen populations from West Africa. Post-graduate seminar, presented as part of the 2nd year Ph.D. progress report, to the Department of Biological Sciences, University of Exeter. (G)
- BANNIZA, S. and HOLDERNESS, M. (1997). Interim Progress Report (Oct. 1996 - Aug. 1997). Copies supplied to West African Rice Development Association, Côte d'Ivoire; Savanna Agricultural Research Institute, Ghana; Crop Research Institute, Ghana; Dr. A. Sy, IITA; Dr. F. Correa-Victoria, CIAT. (G)
- CHIPILI, J., SREENIVASAPRASAD, S., TURNER, H., BLACK, R., BANNIZA, S., HOLDERNESS, M. and SERE, Y. (1998). Identification and characterisation of key screening sites for blast and scald resistance in West Africa. Abstract submitted to the WARDA/NARS-IPM Task Force meeting at WARDA, March 1998. (G)
- SREENIVASAPRASAD, S. and CHIPILI, J. (1997) Interim progress report on the molecular characterisation of the rice blast pathogen. pp. 15. (Oct. 1996 - Aug. 1997). Copies supplied to West African Rice Development Association, Côte d'Ivoire; Savanna Agricultural Research Institute, Ghana; Crop Research Institute, Ghana; Dr. A. Sy, IITA; Dr. F. Correa-Victoria, CIAT. (G)
- SREENIVASAPRASAD, S., CHIPILI, J. and SERE, Y. (1999) Genetic diversity of the blast pathogen population at rice screening sites: West African experience. Invited presentation at the CPP workshop on Rice Crop Protection in Asia, Bangladesh Rice Research Institute, Chaka, 6 - 8 April 1999 - abstract to be included in workshop proceedings. (B)
- TURNER, H. C. (1997) Interim progress report on the molecular and pathotypical analyses of the rice scald pathogen. (Oct. 1996 - Aug. 1997). Copies supplied to West African Rice Development Association, Côte d'Ivoire; Savanna Agricultural Research Institute, Ghana; Crop Research Institute, Ghana; Dr. A. Sy, IITA; Dr. F. Correa-Victoria, CIAT. (G)
- TURNER, H. C., SREENIVASAPRASAD, S. and HOLDERNESS, M. (1998). Invited presentation of project R6738 activities and outputs at the CPP workshop on Rice Crop Protection in Africa, Natural Resources Institute, Chatham, U.K. 7 - 8 December 1998. (B)
- TURNER, H. C. (1999). Publication of *M. albescens* rDNA ITS-region sequences on EMBL international sequence database. (G)
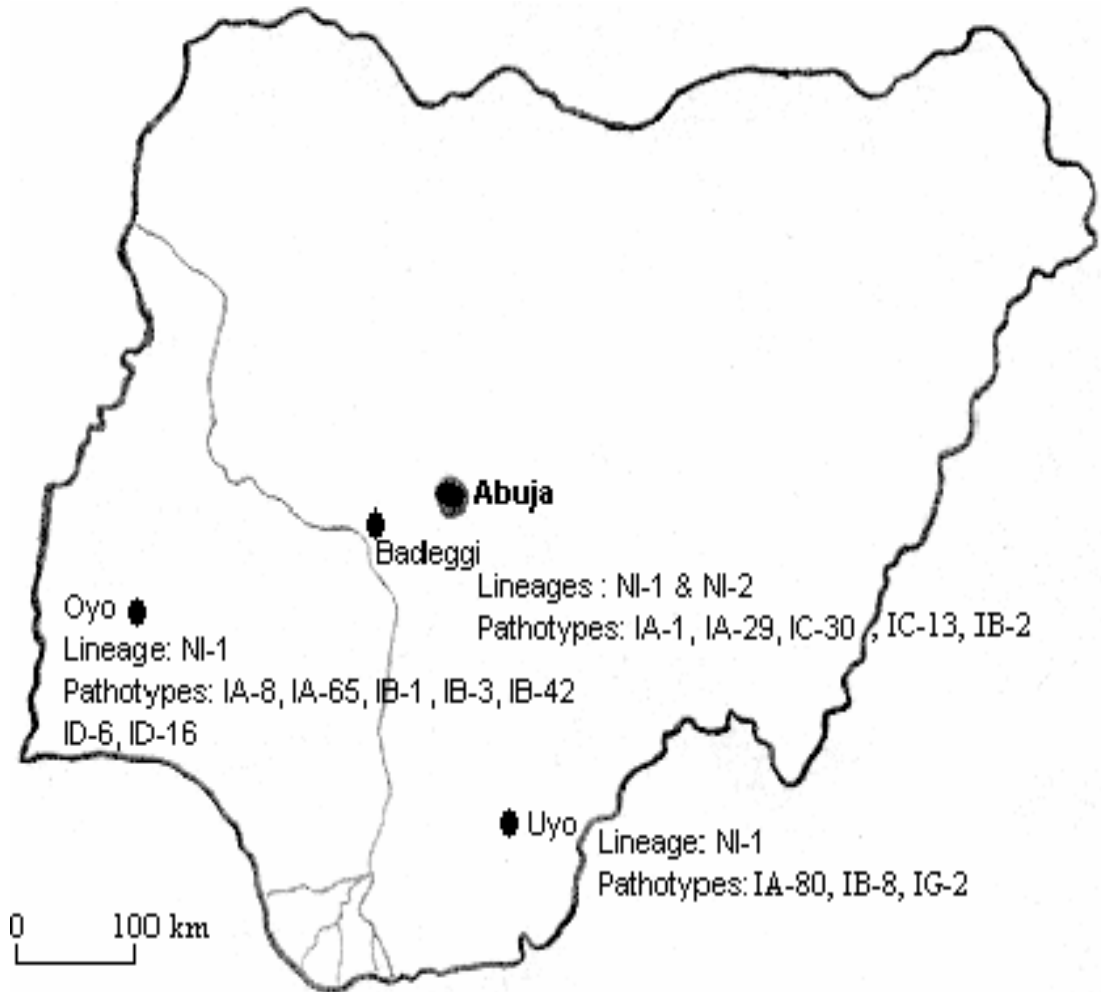- Dissemination of summary report to WARDA, NARS, CIAT is planned.

Vallee du Kou (BF-1)
Farako-Ba (BF-1, 2, 3, 4, 5 and BF-6)
Sideradougou (BF-2, 4, 5 and BF-7)
Banfora (BF-1, 3, 4, 6 and BF-7)
Labola Koumadougou
(BF-1)

PATHOTYPES AT:
Banfora: IA-121, IC-13, IC-22, IC-25, IF-1 and IG-1
Farako-Ba: IA-105, IA-106, IB-9, IC-1, IC-9, IC-10, IC-25, IC-27, IF-1, IG-1, IG-2 and IH-1
Koumadougou: IB-1
Labola: IA-80
Sideradougou: IA-1, IB-38, IC-1, IC-25, IC-31 and IF-2
Vallee du Kou: II-1, IB-32 and IB-64

0   100 km

Figure B1. Diversity and distribution of *M. grisea* lineages and pathotypes in Burkina Faso

Korhogo
Lineages: CD-1 & CD-2
Pathotypes: IC-13, ID-13

Lineage: CD-1
Pathotype: IA-112
Sakassou

Bouake (M'be)
Lineages: CD-2
Pathotype: ID-15

Man
Lineages: CD-1, CD-2, CD-3, CD-4, CD-5
Pathotypes: IA-13, IA-73, IA-80, IB-45, IC-21, ID-9, ID-14

Abidjan

0   100 km

Figure B2. Diversity and distribution of *M. grisea* lineages and pathotypes in Cote d Ivoire

Lineages: GH-1 and GH-3
Pathotypes: IA-88, IB-1, IC-25

ungrouped genotypes:
Pathotypes: IA-13, IC-14, IC-17,
IC-19, IC-25, IC-26, IC-29

Lineages: GH-1 and GH-2
Pathotypes: IA-1, IA-2, IB-1, IB-2, IB-7, IB-9,
IB-13, IB-21, IB-46, IB-61, IC-29 and IG-1

(Kwadaso) ◆ Kumasi
Lineage: GH-1
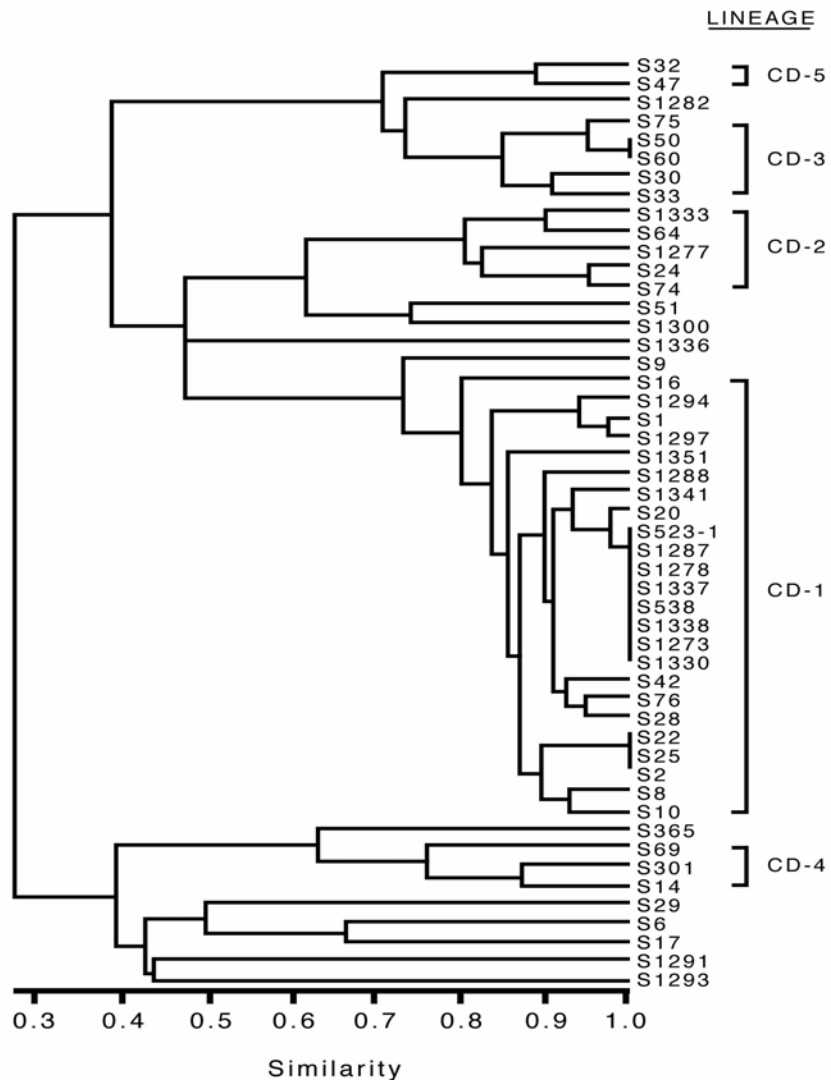Pathotype: ID-14

0   100 km

Figure B3. Diversity and distribution of *M. grisea* lineages and pathotypes in Ghana

Figure B4. Diversity and distribution of *M. grisea* lineages and pathotypes in Nigeria
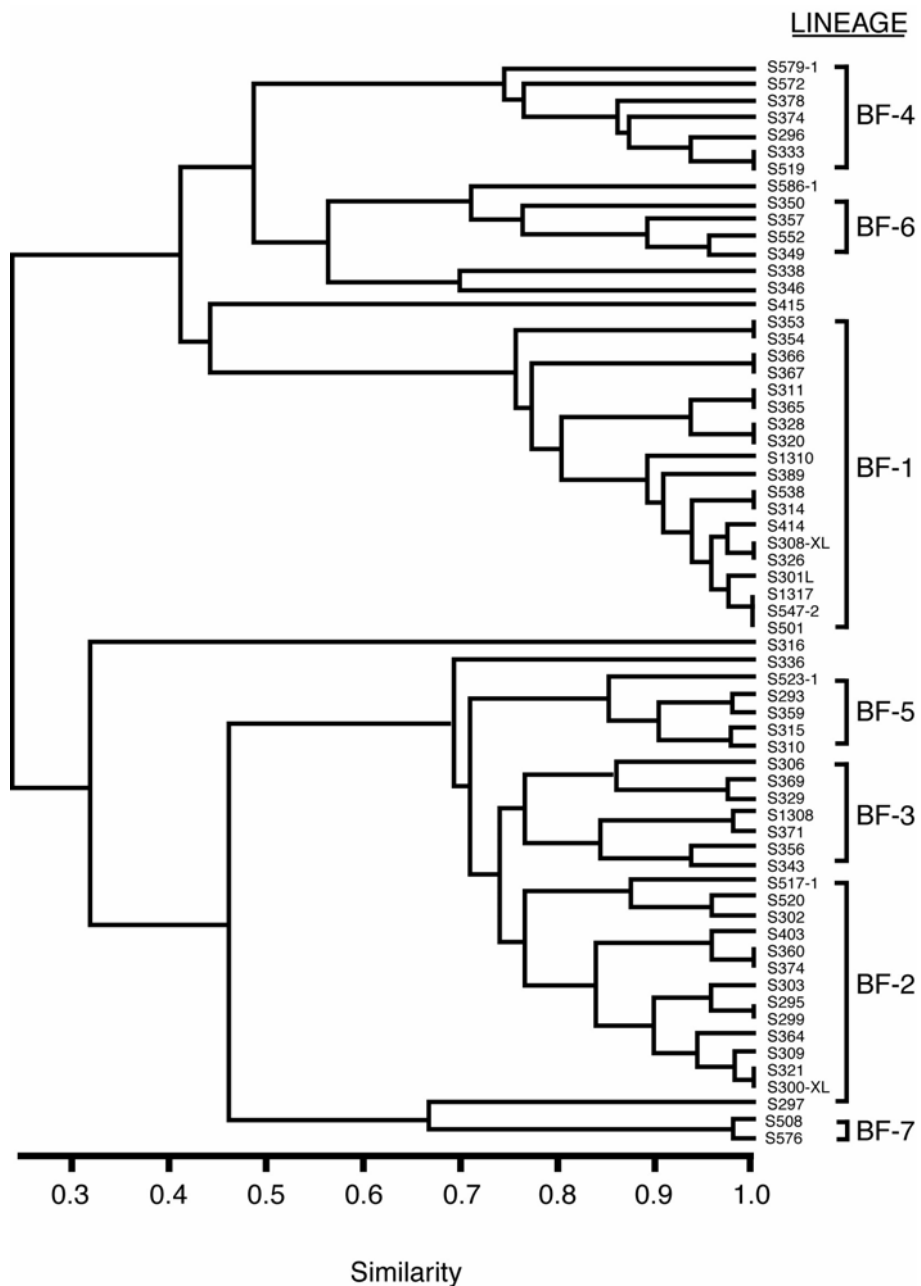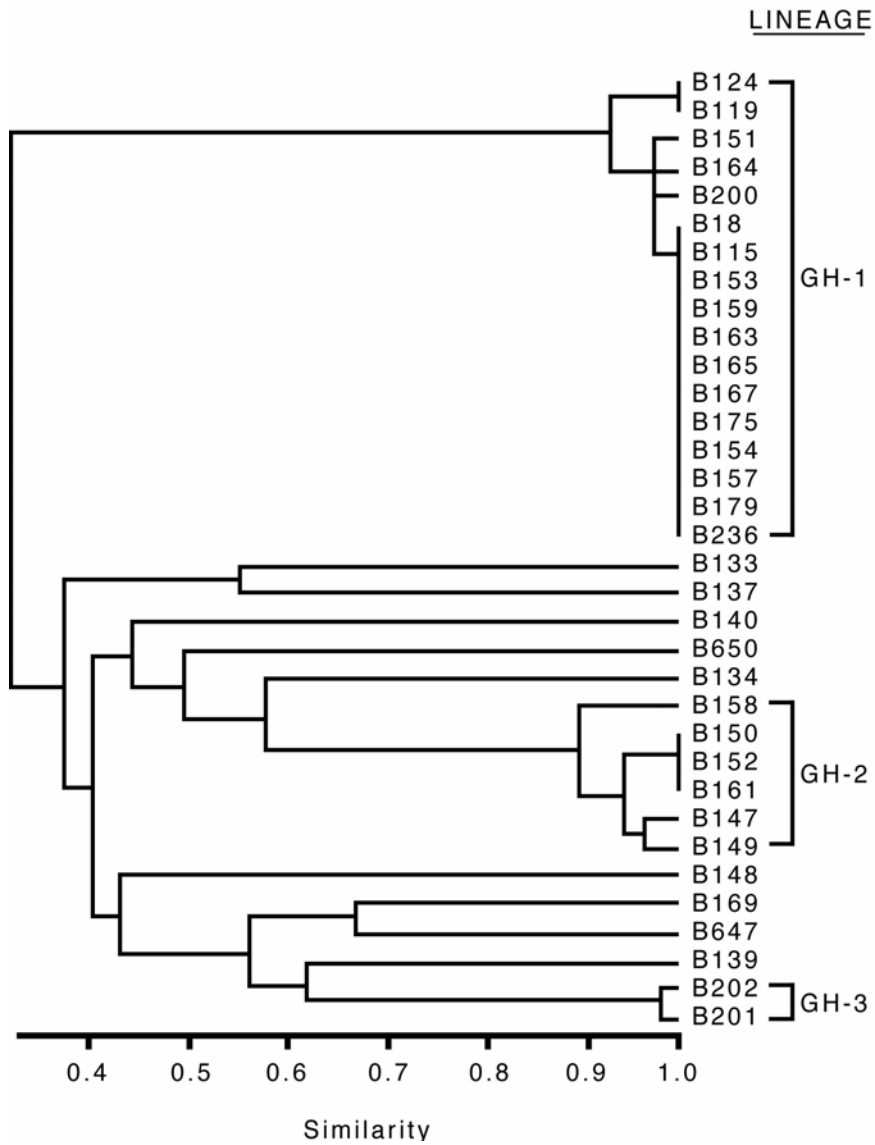
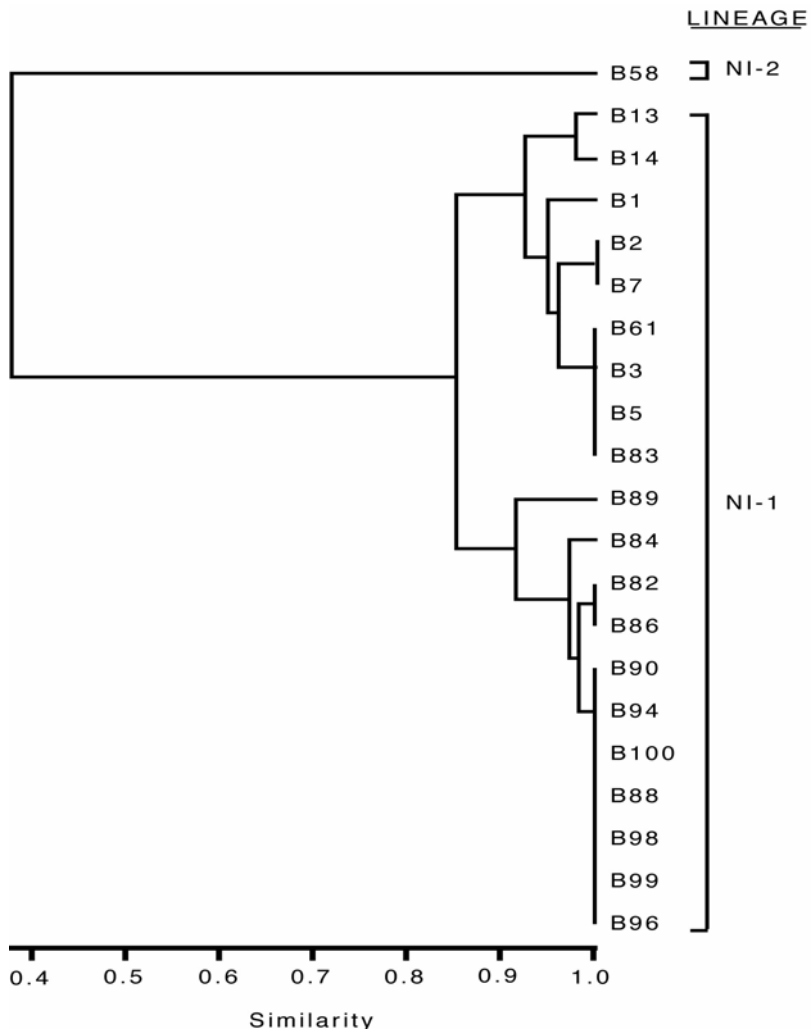**Figure B5.** *M. grisea* lineages in Burkina Faso

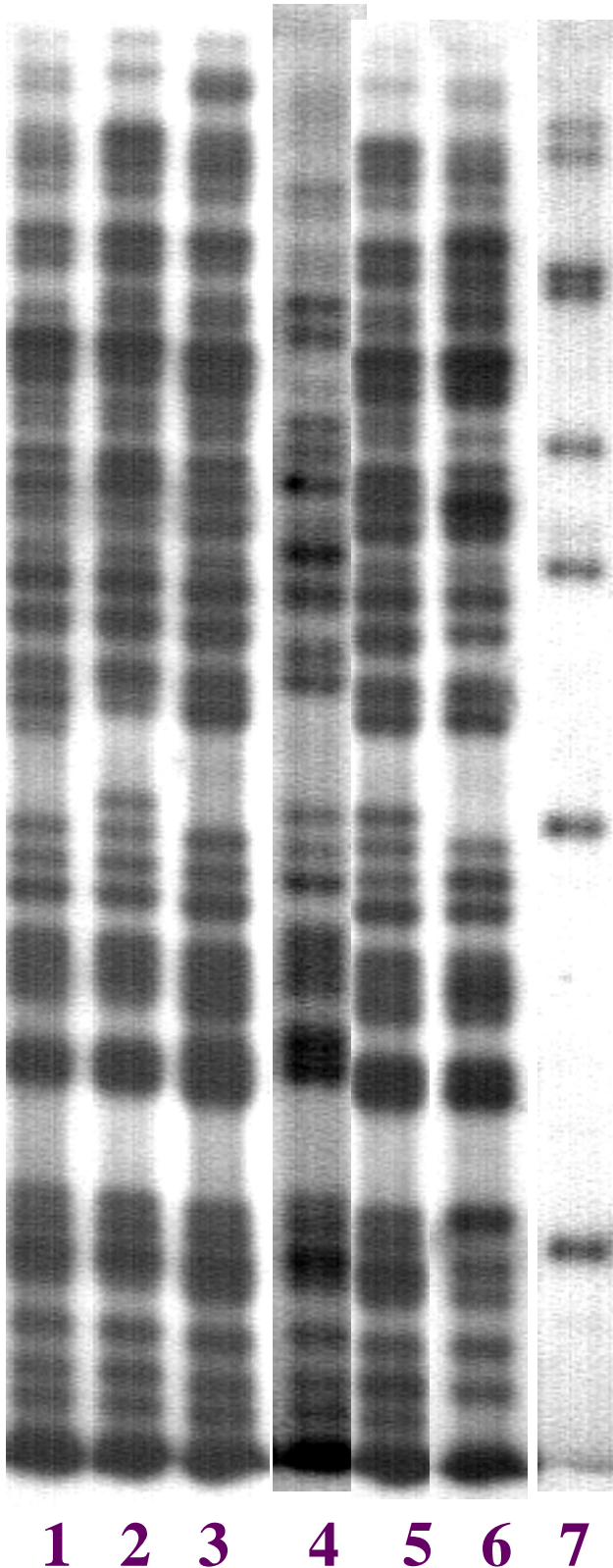**Figure B6.** *M. grisea* lineages in Cote d' Ivoire

Figure B7. *M. grisea* lineages in Ghana

Figure B8. *M. grisea* lineages in Nigeria

Figure B9. MGR586 profiles of seven
M. grisea isolates from Nigeria
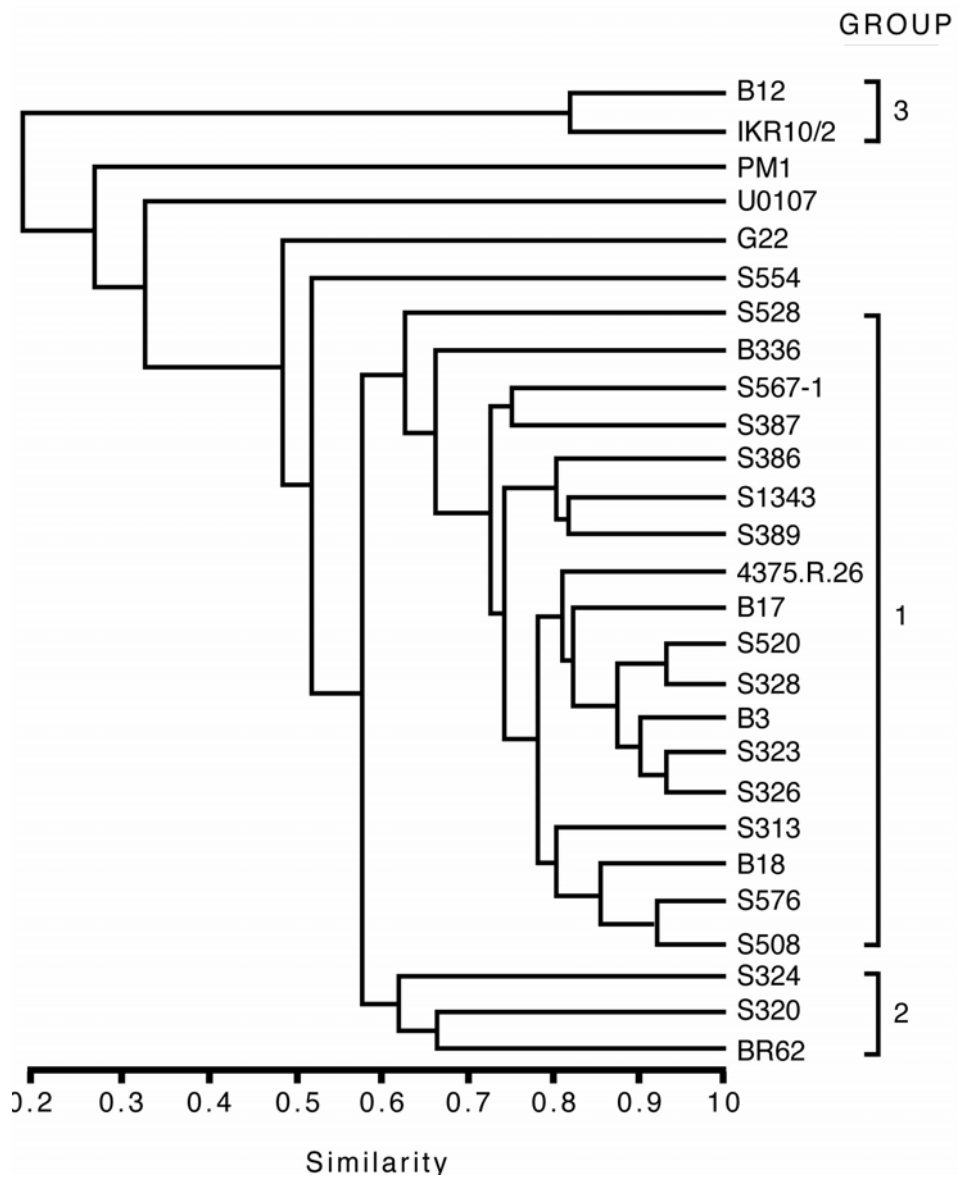

1 2 3 4 5 6 7

Isolates 1 - 6

2 sites - Uyo and Abia

6 different lines/vars.

At least 3 haplotypes
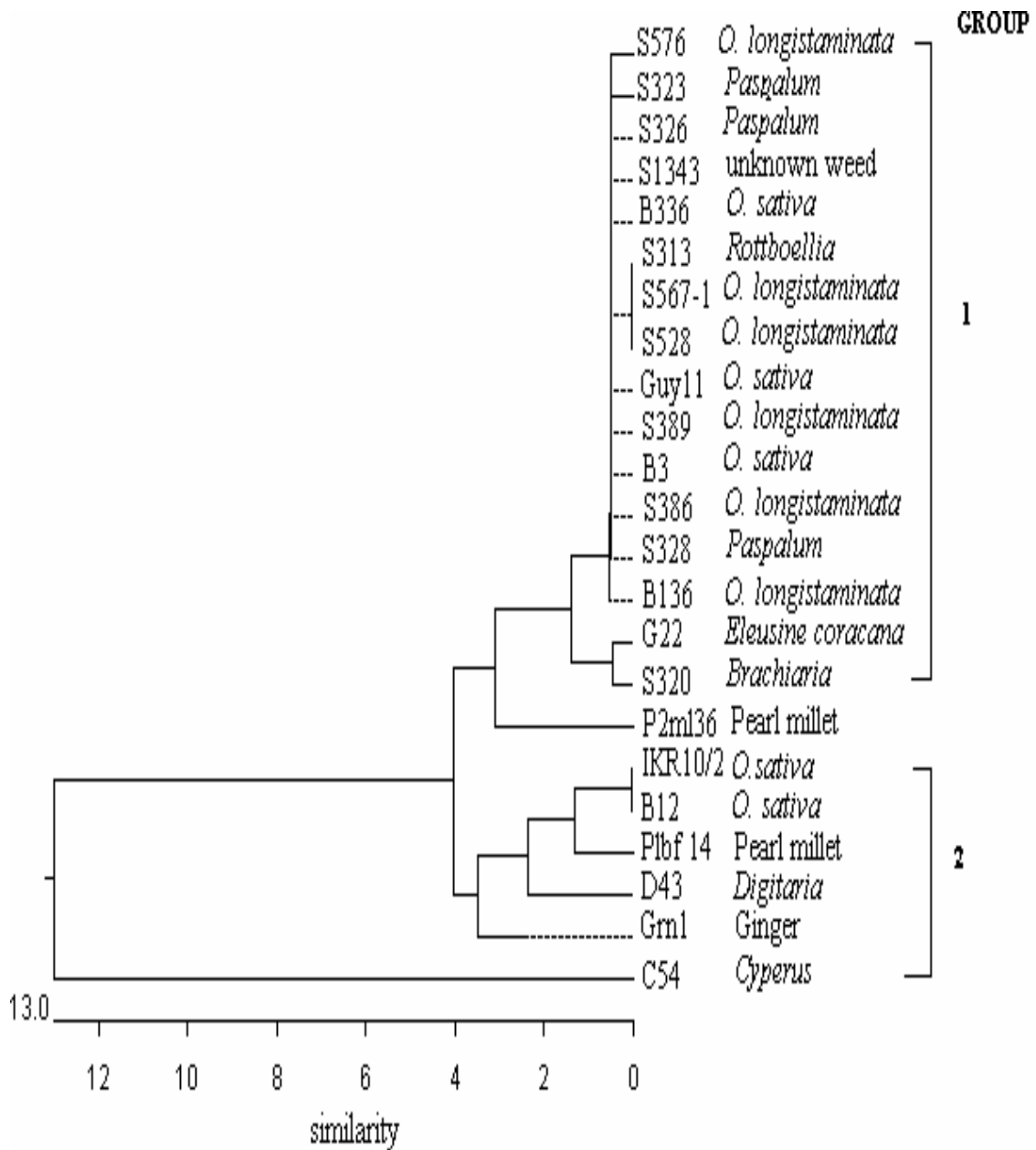
A single lineage

Isolate 7 - atypical
MGR586 fingerprint

Figure B10. RAPD-PCR analysis of atypical *M. grisea* isolates

S576 — *O. longistaminata*
S323 — *Paspalum*
S326 — *Paspalum*
S1343 — unknown weed
B336 — *O. sativa*
S313 — *Rottboellia*
S567-1 — *O. longistaminata*
S528 — *O. longistaminata*
Guy11 — *O. sativa*
S389 — *O. longistaminata*
B3 — *O. sativa*
S386 — *O. longistaminata*
S328 — *Paspalum*
B136 — *O. longistaminata*
G22 — *Eleusine coracana*
S320 — *Brachiaria*
P2ml36 — Pearl millet
IKR10/2 — *O. sativa*
B12 — *O. sativa*
Ptbf 14 — Pearl millet
D43 — *Digitaria*
Grn1 — Ginger
C54 — *Cyperus*

GROUP

1

2

13.0

12   10   8   6   4   2   0

similarity

Figure B11. Relatedness of *M. grisea* isolates from various hosts based on ITS 1 sequence

APPENDIX 5

PUBLICATION OF *M. ALBESCENS* rDNA ITS-REGION SEQUENCES ON EMBL INTERNATIONAL SEQUENCE DATABASE.

Accession#:   **AJ132505**
Status: **CONFIDENTIAL UNTIL 25-FEB-1999**
Description: **Monographella albescens 5.8S rRNA gene and internal transcribed spacer 1   (ITS1) and internal transcribed spacer 2 (ITS2), isolate CIAT 7**

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ID   MAL132505  standard; DNA; FUN; 479 BP.
XX
AC   AJ132505;
XX
SV   AJ132505.1
XX
NI   e1390899
XX
DT   01-MAR-1999 (Rel. 59, Created)
DT   01-MAR-1999 (Rel. 59, Last updated, Version 1)
XX
DE   Monographella albescens 5.8S rRNA gene and internal transcribed
DE   spacer 1
DE   (ITS1) and internal transcribed spacer 2 (ITS2), isolate CIAT 7
XX
KW   5.8S ribosomal RNA; 5.8S rRNA gene; internal transcribed spacer 1;
KW    internal transcribed spacer 2; ITS1; ITS2.
XX
OS   Monographella albescens
OC   Eukaryota; Fungi; Ascomycota; Euascomycetes; Hyponectriaceae;
OC   Monographella.
XX
RN   [1]
RP   1-479
RA   Turner H.C.;
RT   ;
RL   Submitted (25-JAN-1999) to the EMBL/GenBank/DDBJ databases.
RL   Turner H.C., Pest Management, Natural Resources Institute, Central
RL   Avenue, Chatham Maritime, Kent ME4 4TB, UK.
XX
RN   [2]
RA   Turner H.C., Sere Y., Twumasi J., Nutsugah S., Brown A.E., Black R.;
RT   "Molecular variability in W. African populations of the rice leaf scald
RT   pathogen, Monographella albescens";
RL   Unpublished.
XX
FH   Key             Location/Qualifiers
FH
FT   source          1..479
FT                   /organism="Monographella albescens"
FT                   /country="Colombia"
FT                   /db_xref="taxon:87749"
FT                   /isolate="CIAT 7"
FT                   /cell_type="mycelium"
FT   misc_feature    1..148
FT                   /note="internal transcribed spacer 1, ITS1"
FT   rRNA            149..306
FT                   /gene="5.8S rRNA"
FT                   /product="5.8S ribosomal RNA"
FT   misc_feature    307..479
FT                   /note="internal transcribed spacer 2, ITS2"
XX
SQ   Sequence 479 BP; 122 A; 127 C; 106 G; 124 T; 0 other;
     AGTTAACAAC TCTCCAAACC ATGTGAACTT ACCACTGTTG CCTCGGTGGT TGGCGCTCCT        60
     CCCTCTCCGA AAGGGGCGCC GCCGCCGGCG GACAAACTAA ACTCTTGTCA ACTTTGTCAA       120
     ATCTGAATCT AAACTAAGAA ATAAGTTAAA ACTTTCAACA ACGGATCTCT TGGTTCTGGC       180
     ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA       240
     TCGAATCTTT GAACGCACAT TGCGCCCATT AGTATTCTAG TGGGCATGCC TGTTCGAGCG       300
     TCATTTCAAC CCTTAAGCCT AGCTTAGTGT TGGGAGACTG CGCTAAACCG CAGCTCCTCA       360
     AAACCAGTGG CGGAGTCCTC TGTGCTCTGA GCGTAGTAAT TCTCTATCTC GCTTGTATGA       420
     ACGCAGTGGT CGACGGCCAT AAACCGCACC TCTCCCCCTC CAGGGATTGG GCACCTTTT        479
```

Accession#:   **AJ132506**
Status: **CONFIDENTIAL UNTIL 25-FEB-1999**
Description: **Monographella albescens 5.8S rRNA gene and internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), isolate CIAT 11**

ID   MAL132506 standard; DNA; FUN; 465 BP.
XX
AC   AJ132506;
XX
SV   AJ132506.1
XX
NI   e1390900
XX
DT   01-MAR-1999 (Rel. 59, Created)
DT   01-MAR-1999 (Rel. 59, Last updated, Version 1)
XX
DE   Monographella albescens 5.8S rRNA gene and internal transcribed
DE   spacer 1
DE   (ITS1) and internal transcribed spacer 2 (ITS2), isolate CIAT 11
XX
KW   5.8S ribosomal RNA; 5.8S rRNA gene; internal transcribed spacer 1;
KW   internal transcribed spacer 2; ITS1; ITS2.
XX
OS   Monographella albescens
OC   Eukaryota; Fungi; Ascomycota; Euascomycetes; Hyponectriaceae;
OC   Monographella.
XX
RN   [1]
RP   1-465
RA   Turner H.C.;
RT   ;
RL   Submitted (25-JAN-1999) to the EMBL/GenBank/DDBJ databases.
RL   Turner H.C., Pest Management, Natural Resources Institute, Central
RL   Avenue, Chatham Maritime, Kent ME4 4TB, UK.
XX
RN   [2]
RA   Turner H.C., Sere Y., Twumasi J., Nutsugah S., Brown A.E., Black R.;
RT   "Molecular variability in W. African populations of the rice leaf scald
RT   pathogen, Monographella albescens";
RL   Unpublished.
XX
FH   Key   Location/Qualifiers
FH
FT   source   1..465
FT   /organism="Monographella albescens"
FT   /country="Colombia"
FT   /db_xref="taxon:87749"
FT   /isolate="CIAT 11"
FT   /cell_type="mycelium"
FT   misc_feature   1..134
FT   /note="internal transcribed spacer 1, ITS1"
FT   rRNA   135..292
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FT   /product="5.8S ribosomal RNA"
FT   misc_feature   292..465
FT   /note="internal transcribed spacer 2, ITS2"
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SQ   Sequence 465 BP; 116 A; 123 C; 106 G; 120 T; 0 other;
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    GGCGCCGCCG CCGGCGGACA AACTAAACTC TTGTCAACTT TGTCAAATCT GAATCTAAAC   120
    TAAGAAATAA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGGCATCG ATGAAGAACG   180
    CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC   240
    GCACATTGCG CCCATTAGTA TTCTAGTGGG CATGCCTGTT CGAGCGTCAT TTCAACCCTT   300
    AAGCCTAGCT TAGTGTTGGG AGACTGCGCT AAACCGCAGC TCCTCAAAAC CAGTGGCGGA   360
    GTCCTCTGTG CTCTGAGCGT AGTAATTCTC TATCTCGCTT GTATGAACGC AGCGGTCGAC   420
    GGCCATAAAC CGCGCCTCTC CCCCTCCAGG GATTGGGCAC CTTTT   465

Accession#: AJ132507
Status: CONFIDENTIAL UNTIL 25-FEB-1999
Description: Monographella albescens 5.8S rRNA gene and internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 ITS2), isolate=S.1.1

ID   MAL132507 standard; DNA; FUN; 470 BP.
XX
AC   AJ132507;
XX
SV   AJ132507.1
XX
NI   e1390901
XX
DT   01-MAR-1999 (Rel. 59, Created)
DT   01-MAR-1999 (Rel. 59, Last updated, Version 1)
XX
DE   Monographella albescens 5.8S rRNA gene and internal transcribed
DE   spacer 1
DE   (ITS1) and internal transcribed spacer 2 ITS2), isolate=S.1.1
XX
KW   5.8S ribosomal RNA; 5.8S rRNA gene; internal transcribed spacer 1;
KW   internal transcribed spacer 2; ITS1; ITS2.
XX
OS   Monographella albescens
OC   Eukaryota; Fungi; Ascomycota; Euascomycetes; Hyponectriaceae;
OC   Monographella.
XX
RN   [1]
RP   1-470
RA   Turner H.C.;
RT   ;
RL   Submitted (25-JAN-1999) to the EMBL/GenBank/DDBJ databases.
RL   Turner H.C., Pest Management, Natural Resources Institute, Central
RL   Avenue, Chatham Maritime, Kent ME4 4TB, UK.
XX
RN   [2]
RA   Turner H.C., Sere Y., Twumasi J., Nutsugah S., Brown A.E., Black R.;
RT   "Molecular variability in W. African populations of the rice leaf scald
RT   pathogen, Monographella albescens";
RL   Unpublished.
XX
FH   Key   Location/Qualifiers
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FT   source   1..470
FT   /organism="Monographella albescens"
FT   /country="Nigeria"
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FT   /isolate="S.1.1"
FT   /cell_type="mycelium"
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FT   /note="internal transcribed spacer 1, ITS1"
FT   rRNA   139..296
FT   /gene="5.8S rRNA"
FT   /product="5.8S ribosomal RNA"
FT   misc_feature   296..470
FT   /note="internal transcribed spacer 2, ITS2"
XX
SQ   Sequence 470 BP; 117 A; 124 C; 106 G; 123 T; 0 other;
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     AAGGGGCGCC GCCGCCGGCG GACAAACTAA ACTCTTGTCA ACTTTGTCAA ATCTGAATCT   120
     AAACTAAGAA ATAAGTTAAA ACTTTCAACA ACGGATCTCT TGGTTCTGGC ATCGATGAAG   180
     AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT   240
     GAACGCACAT TGCGCCCATT AGTATTCTAG TGGGCATGCC TGTTCGAGCG TCATTTCAAC   300
     CCTTAAGCCT AGCTTAGTGT TGGGAGACTG CGCTAAACCG CAGCTCCTCA AAACCAGTGG   360
     CGGAGTCGCT CTGTGCTCTG AGCGTAGTAA TTCTCTATCT CGCTTGTATG AACGCAGTGG   420
     TCGACGGCCA TAAACCGCAC CTCTCCCCCT CCAGGGATTG GGCACCTTTT   470

Accession#: AJ132508
Status: CONFIDENTIAL UNTIL 25-FEB-1999
Description: Monographella albescens 5.8S rRNA gene and internal transcribed spacer 1 (ITS1) and internal
transcribed spacer 2 ITS2), isolate S.11.1

ID   MAL132508 standard; DNA; FUN; 471 BP.
XX
AC   AJ132508;
XX
SV   AJ132508.1
XX
NI   e1390902
XX
DT   01-MAR-1999 (Rel. 59, Created)
DT   01-MAR-1999 (Rel. 59, Last updated, Version 1)
XX
DE   Monographella albescens 5.8S rRNA gene and internal transcribed
DE   spacer 1
DE   (ITS1) and internal transcribed spacer 2 ITS2), isolate S.11.1
XX
KW   5.8S ribosomal RNA; 5.8S rRNA gene; internal transcribed spacer 1;
KW   internal transcribed spacer 2; ITS1; ITS2.
XX
OS   Monographella albescens
OC   Eukaryota; Fungi; Ascomycota; Euascomycetes; Hyponectriaceae;
OC   Monographella.
XX
RN   [1]
RP   1-471
RA   Turner H.C.;
RT   ;
RL   Submitted (25-JAN-1999) to the EMBL/GenBank/DDBJ databases.
RL   Turner H.C., Pest Management, Natural Resources Institute, Central
RL   Avenue, Chatham Maritime, Kent ME4 4TB, UK.
XX
RN   [2]
RA   Turner H.C., Sere Y., Twumasi J., Nutsugah S., Brown A.E., Black R.;
RT   "Molecular variability in W. African populations of the rice leaf scald
RT   pathogen, Monographella albescens";
RL   Unpublished.
XX
FH   Key   Location/Qualifiers
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FT   source   1..471
FT   /organism="Monographella albescens"
FT   /country="Nigeria"
FT   /db_xref="taxon:87749"
FT   /isolate="S.11.1"
FT   /cell_type="mycelium"
FT   misc_feature   1..139
FT   /note="internal transcribed spacer 1, ITS1"
FT   rRNA   140..297
FT   /gene="5.8S rRNA"
FT   /product="5.8S ribosomal RNA"
FT   misc_feature   298..471
FT   /note="internal transcribed spacer 2, ITS2"
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SQ   Sequence 471 BP; 117 A; 125 C; 106 G; 123 T; 0 other;
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     AAAGGGGCGC CGCCGCCGGC GGACAAACTA AACTCTTGTC AACTTTGTCA AATCTGAATC   120
     TAAACTAAGA AATAAGTTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG CATCGATGAA   180
     GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT   240
     TGAACGCACA TTGCGCCCAT TAGTATTCTA GTGGGCATGC CTGTTCGAGC GTCATTTCAA   300
     CCCTTAAGCC TAGCTTAGTG TTGGGAGACT GCGCTAAACC GCAGCTCCTC AAAACCAGTG   360
     GCGGAGTCGC TCTGTGCTCT GAGCGTAGTA ATTCTCTATC TCGCTTGTAT GAACGCAGTG   420
     GTCGACGGCC ATAAACCGCA CCTCTCCCCC TCCAGGGATT GGGCACCTTT T   471

Accession#:   **AJ132509**
Status: **CONFIDENTIAL UNTIL 25-FEB-1999**
Description: **Monographella albescens 5.8S rRNA gene and internal transcribed spacer 1   (ITS1) and internal transcribed spacer 2 ITS2), isolate S.12.1**

ID   MAL132509 standard; DNA; FUN; 472 BP.
XX
AC   AJ132509;
XX
SV   AJ132509.1
XX
NI   e1390903
XX
DT   01-MAR-1999 (Rel. 59, Created)
DT   01-MAR-1999 (Rel. 59, Last updated, Version 1)
XX
DE   Monographella albescens 5.8S rRNA gene and internal transcribed
DE   spacer 1
DE   (ITS1) and internal transcribed spacer 2 ITS2), isolate S.12.1
XX
KW   5.8S ribosomal RNA; 5.8S rRNA gene; internal transcribed spacer 1;
KW   internal transcribed spacer 2; ITS1; ITS2.
XX
OS   Monographella albescens
OC   Eukaryota; Fungi; Ascomycota; Euascomycetes; Hyponectriaceae;
OC   Monographella.
XX
RN   [1]
RP   1-472
RA   Turner H.C.;
RT   ;
RL   Submitted (25-JAN-1999) to the EMBL/GenBank/DDBJ databases.
RL   Turner H.C., Pest Management, Natural Resources Institute, Central
RL   Avenue, Chatham Maritime, Kent ME4 4TB, UK.
XX
RN   [2]
RA   Turner H.C., Sere Y., Twumasi J., Nutsugah S., Brown A.E., Black R.;
RT   "Molecular variability in W. African populations of the rice leaf scald
RT   pathogen, Monographella albescens";
RL   Unpublished.
XX
FH   Key   Location/Qualifiers
FH
FT   source   1..472
FT   /organism="Monographella albescens"
FT   /country="Nigeria"
FT   /db_xref="taxon:87749"
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FT   /product="5.8S ribosomal RNA"
FT   misc_feature   300..472
FT   /note="internal transcribed spacer 2, ITS2"
XX
SQ   Sequence 472 BP; 118 A; 125 C; 106 G; 123 T; 0 other;
     AACTCTCCAA ACCATGTGAA CTTACCACTG TTGCCTCGGT GGTTGGCGCT CCTCCCTCTC   60
     TGAAAGGGGC GCCGCCGCCG GCGGACAAAC TAAACTCTTG TCAACTTTGT CAAATCTGAA   120
     TCTAAACTAA GAAATAAGTT AAAACTTTCA ACAACGGATC TCTTGGTTCT GGCATCGATG   180
     AAGAACGCAG CGAAATGCGA TAAGTAATGT GAATTGCAGA ATTCAGTGAA TCATCGAATC   240
     TTTGAACGCA CATTGCGCCC ATTAGTATTC TAGTGGGCAT GCCTGTTCGA GCGTCATTTC   300
     AACCCTTAAG CCTAGCTTAG TGTTGGGAGA CTGCGCTAAA CCGCAGCTCC TCAAAACCAG   360
     TGGCGGAGTC CTCTGTGCTC TGAGCGTAGT AATTCTCTAT CTCGCTTGTA TGAACGCAGT   420
     GGTCGACGGC CATAAACCGC GCCTCTCCCC CTCCAGGGAT TGGGCACCTT TT   472

MOLECULAR VARIABILITY OF THE RICE LEAF SCALD PATHOGEN, *MONOGRAPHELLA ALBESCENS*

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Background

Rice leaf scald is a common disease of rice crops, occurring in nearly all of the rice-growing regions of the world. The causal organism, *Monographella albescens* (Thüm) can pose a serious threat to rice production under favourable climatic and cultural conditions, particularly in upland areas. However, almost nothing is known about the variability of *M. albescens* within or between populations [1]. We present here an analysis of the molecular variability of isolates collected predominantly from West Africa, with some additional material from Colombia and the Philippines, and discuss the implications for the evolution and spread of the pathogen.

Materials and methods

Samples of scald-infected rice leaves (Fig. 1) were obtained from trial sites run by the West African Rice Development Association (WARDA). Non-African isolates were provided by the International Rice Research Institute (IRRI) and the Centro Internationale de Agricultura Tropicale (CIAT). DNA was extracted from isolates of the pathogen, and subjected to molecular analyses. The ribosomal DNA (rDNA) and intergenic spacer (IGS) regions were each amplified by PCR and restriction fragment polymorphisms in these two regions determined, using eight and six restriction nucleases respectively. The data were analysed through Unweighted Pair-Group Method, Arithmetic averages (UPGMA) cluster analysis under NT-SYS. Additionally, rDNA from selected isolates was amplified using ITS1 and ITS4 primers [2], and the products sequenced at the Queen's University of Belfast. These sequences were then aligned and compared using DNAStar software.

Results and discussion

Of eight restriction enzymes tested, only one (Hha I) revealed any variation in the rDNA region of *M. albescens*, with just two restriction fragment patterns being observed in over 250 isolates. Sequencing of the PCR-amplified rDNA region from 5 isolates, selected from both of these groups, confirmed the very low level of variability. Comparison of the sequences (Fig. 2) showed the presence of just four single-base variations within the c.480bp PCR products. Restriction sites for Bbv I and Fnu 4HI were identified overlapping two of the variable bases in the ITS 2 region, but use of these enzymes resulted in too many fragments, of too small a size, to be of use in further analysis of this region.

Consequently, primers PN 11 and PN 22 were used to amplify a c4.5Kb product from the IGS region. Restriction analysis of this larger PCR product, using six nucleases, revealed a greater range of fingerprints which were analysed as described above. The resultant dendrogram (Fig. 3) shows that many of the isolates examined produce identical fingerprints with all six nucleases. However, a greater degree of variation is observed than in the ITS region. With the exception of one isolate, the two major groupings shown in Figure 3 correlate exactly to the two ITS fingerprints observed for these same isolates.

The dendrogram (Fig. 3) suggests a geographical bias, in that only one of the isolates from Ogun, Nigeria, falls within the lower of the two main clusters, although isolates from the other two Nigerian sites are relatively evenly divided. However, ongoing analyses of a second, larger set of samples are beginning to suggest that the observed bias in isolates from Ogun is an artefact of the limited number of samples collected in that cropping season. It is also worth noting that multiple isolations from a single lesion (single-tip isolations are

routinely made) can yield isolates belonging to different groupings as identified from IGS or ITS restriction fingerprints, indicating that what appears to be one lesion may result from infection by more than one individual.

Our findings to date suggest that *M. albescens*, which is nearly ubiquitous in rice-growing regions around the world, shows a low level of variability at the molecular level. This may be indicative of the wider population having developed from a very limited original source, probably through the movement of germplasm between regions. Since *M. albescens* is known to infect non-rice hosts (e.g. *Echinochloa crus-galli* [3]), it would be interesting to also compare isolates from different hosts at the molecular level.

Figure 1: Typical symptoms of rice leaf scald on material collected in W. Africa. Lesions generally progress from leaf tips and margins, developing characteristic light-and-dark striations.

Figure 2: Nucleotide sequences of ribosomal DNA, including the ITS 1, 5.8s and ITS 2 regions, from selected isolates of *M. albescens*.

Figure 3: Dendrogram showing the clustering of *M. albescens* isolates on the basis of RFLPs within the IGS region. Each square represents a single isolate, with colour-codes representing the sites of origin. The dendrogram was generated from restriction fragment polymorphisms within a PCR-amplified product, using UPGMA cluster analysis with NT-SYS software.

Key words:
Rice, leaf scald, *Monographella albescens, Gerlachia oryzae, Rhynchosporium oryzae*, molecular variability, West Africa.

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A0801 / ZA0266 File Note: Workshop on Rice Crop Protection in Africa, linking with PSRP workshop on Rice Biotechnology: 8-10 December 1998

Background

1. Crop Protection Programme (CPP) funding was used to bring together African and UK scientists (listed in Annex I) with an interest in rice crop production and protection in Africa, with the objective of identifying, through the Workshop on Rice Crop Protection in Africa (NRI, 8[th] December), those areas of research where CPP-sponsored activities will have the greatest potential impact on poverty alleviation. Delegates were also invited to the Plant Sciences Research Programme (PSRP) Workshop on Rice Biotechnology (De Montfort University, 9[th]-10[th] December), where presentations were made on current PSRP activities. The potential for linkages between PSRP and CPP activities, and African institutions was explored. A schedule of presentations and discussion sessions is given in Annex II.

2. Proceedings of the workshops are in preparation, and will be published in booklet format for dissemination to workshop participants and other interested parties early in 1999. A summary of the main points identified during the workshops is presented below.

3. **Rice Crop Protection Workshop**
   Country reports:
   Reports on rice production systems, and current and emerging crop protection constraints, were presented by Dr. Kanyeka (Tanzania), Dr. Twumasi (Ghana) and Dr. Kyetere (Uganda). Dr. Ukwungu (Nigeria) and Dr Gaudreau (IRRI-Madagascar) were unable to attend, but will be providing information in due course. The specifics of the country reports will be presented in the workshop proceedings, but overall conclusions are presented below:

- In all reporting countries, rice production is being intensified.
- Local rice production cannot always compete with imported rice
- Intensification is resulting in an increasing economic significance of production constraints, particularly of pests and diseases
- Weeds are one of the most serious constraints to rice production
- Main pests across the countries include:
    ◊ Birds
    ◊ Rodents
    ◊ African rice gall midge
    ◊ Stem borers
    ◊ Hispa beetle
- Main diseases across the countries include:
    ◊ RYMV
    ◊ Blast
    ◊ Brown spot
    ◊ Bacterial leaf streak
    ◊ Sheath rots and blights
- Declining soil fertility, commonly arising from inadequate land management strategies
- Poor availability of good quality seeds, particularly of improved cultivars adapted to local conditions and low-input systems
- Poor post-harvest processing
- Poor water management

4. **WARDA reports:**

WEEDS: Weeds comprise a major constraint to rice production, particularly where labour availability is low. WARDA is addressing this issue through such initiatives as the interspecific hybrid programme (production of varieties adapted to compete with weeds), and testing of leguminous cover crops to both improve soil nitrogen levels and reduce weed-seed build-up. More information is needed on various aspects of weed ecology: weed seedbanks, dormancy periods, effects of burial and flooding, etc. In particular, the shift in weed populations and ecologies in response to changing cultural practices, due to rice production intensification, and their implications, are not currently being addressed.

DISEASES: WARDA has adopted 2 main approaches. The first is to develop an integrated research thrust. The second is to adopt strategically-oriented projects, for example targeting blast and RYMV. The objective is to develop ecosystem-specific resistance donors and IPM strategies for control.

Blast research is being directed along several lines. Biological characterisation (virulence, mating type) and assessment of molecular variability of the pathogen is being conducted, along with mapping of type distributions and study of the effect of environmental and host factors on phenotype distribution. CPP is funding a significant contribution to the early stages of this work, under a project due to complete in March '99. Further WARDA objectives include the identification and characterisation of horizontal resistance, and the development of a set of differential cultivars suitable for use in W. Africa. WARDA will also develop tools for epidemic modelling and management.

RYMV research is currently directed towards increasing the basic knowledge of this disease, and development of appropriate management strategies. Resistance has been identified in some interspecific hybrid progeny, and in a number of indica lines. Assessment of these lines and their use as donors in resistance breeding programs is anticipated. Transgenic material, currently being developed under the DFID PSRP, may also prove useful to this programme.

WARDA has identified a need to look at developing partnerships (e.g. with the E. / C. African rice network, ASARECA), assessment of local situations, and training of farmers and scientists, to equip them to make effective use of new technologies and materials.

BREEDING: To date, gains in the rainfed systems have been limited, due to poor uptake of new varieties. This has been due to their limited tolerance to various biotic and abiotic stresses. Also, despite an on-station yield advantage, many improved varieties perform less well than traditional varieties under low-input conditions. The primary aim of WARDA in recent years has been to develop varieties that outperform traditional varieties under low management levels but will also respond well to additional inputs and improved management. WARDA hope to improve cultivar resistance to blast, RYMV, sheath rot, brown spot, leaf scald, insects and a number of abiotic stresses. Farmer-participatory selection and breeding is being increasingly used as a part of this programme and through a PSRP project.

5.   Current CPP initiatives and areas requiring further attention:

Weeds: WARDA is currently addressing; the development of rice varieties with *high weed suppression / competitiveness* traits, notably through the inter-specific hybrid programme; improved understanding of *weed ecology*; assessment of *intervention methods* (herbicides); development of *modelling and decision support systems*.

Competitive research facility funding for the NRI/WARDA weed scientist, Dr D Johnson.
CPP-funded projects due to start next year including 2 CPP-funded projects in collaboration with WARDA and IRRI. Dr D Johnson also distributed copies of the recently published 'Weeds of Rice in West Africa' (WARDA/DFID/CTA) to the overseas delegates and management team.

Blast: **CPP project R6738 Identification & characterisation of key screening sites for blast/scald resistance in W. Africa**. This is an ongoing rice production constraint, and likely to increase in significance with the intensification of rice production across Africa. There are many international initiatives, particularly in Asia and the USA, which have the potential to benefit the situation in Africa, but more information is needed on the unique specifics of the African situation. **It is anticipated that the CPP blast and scald screening site project will continue to develop collaboration with WARDA**.

RYMV: **R 6763 The epidemiology and control of rice yellow mottle disease in Tanzania.**The economic significance of this disease is increasing with rice production intensification, and resistance material has only been identified very recently. However, some progeny from WARDA's interspecific hybrid material shows good resistance, and a limited number of resistance *indica* cultivars (e.g. Gigante) have now been identified. ORSTOM are looking for molecular markers for RYMV resistance in these lines and the PSRP is also contributing to the development of resistant transgenic material. Initial containment trials of transgenic plants will be started in Côte d'Ivoire once appropriate national biosafety legislation has been approved and construction of containment facilities completed. Development of resistant materials and effective deployment strategies would be enhanced by improved linkages with other African networks, notably ASARECA, as well as direct links with NARS in countries such as Tanzania. **A second phase of the CPP-RYMV epidemiology project should investigate the development of a formal collaborative link with WARDA in facilitating testing of these materials in Tanzania as well as aspects of the life history of vector beetle species.**

Brown spot: No CPP project.This is particularly a problem of low-input systems and is often exacerbated by water-stress. There are no major initiatives in progress. **Significant impact could be achieved by improving seed health measures, through adaptive research. This constraint is an excellent candidate for achieving wide impact through farmer-participatory research.**

African rice gall midge (ARGM): **No CPP project, but potential for CRF through CABI Bioscience.** An increasing problem with intensification of rice production. IRRI has been working on control of the Asian gall midge, with ICGEB and other Indian labs, but their approaches may provide useful leads for work in Africa. An entomologist Dr. C. Williams (CABI Bioscience) based at IITA/WARDA was funded by the DFID Competitive Research Facility (Holdback) to develop management strategies for *Orseolia oryzivora* (07/93-03/97). A visiting scientist, Dr F.Nwilene is now working on this problem at WARDA. **The potential for collaboration on the development of biological control and its incorporation into an IPM project exists through the**

CPP. Again contacts should be made with WARDA to assess their research needs and the development of a WARDA-CPP collaborative project.

Birds and rodents: WARDA and IRRI need to strengthen links in this area. IRRI is currently investigating field and landscape management practices, for example the contribution of weedy field margins (retained, perhaps, as a reservoir for natural predators for biocontrol of insect pests) towards rodent depredation. More information is needed on current initiatives in this area.

Stem borer: This is an emerging problem, again in response to rice production intensification, but still some way from reaching economic significance.

Nematodes: A recently completed CPP project has carried out a comprehensive investigation of the distribution of plant pathogenic nematodes on rice in West Africa, their effects on crop yields under different experimental conditions and to a limited extent on farm. WARDA have previously indicated that they regard nematode pests as important but not among the top priorities for their limited resources, and nematode problems were given relatively low priority in lowland rainfed rice systems by workshop participants.

6. **Workshop conclusions:**
It was agreed that, in both West and East Africa, there is a marked **intensification** of rice production, in response to increasing demand. This intensification is resulting in major changes in the prevalent production constraints, and it is these constraints that urgently need to be addressed.

A **multi-disciplinary, integrated approach** needs to be developed to addressing rice production constraints, notably downstream, adaptive research directed towards development of integrated management of crops in rainfed lowlands.

Strategies for effective deployment of pest management tools need to be developed, in order to ensure their long-term stability.

There is also a need to incorporate capacity-building for biotechnology uptake into projects, to enable countries to benefit from and adopt such technologies. The **collaborative exchange of biotechnologies** is viewed as being of major importance.

Primary **system** focus:
The rainfed lowland system was identified as the primary focus, having the greatest potential for achievable impact.

Primary **constraints** to be addressed:
◊ Weeds
◊ RYMV
◊ Blast (the importance of this in E.Africa, at least, was questioned)
◊ African rice gall midge (ARGM)
◊ Nematodes ? (should be addressed as part of new IPM programs, but is a less visible and less well understood constraint than the above).
Table 3 below gives more detailed information of constraint significance scoring as determined through workshop discussions.

Development and strengthening of **linkages** between interested parties:
As indicated in table 4, a number of useful linkages already exist which could maximise impact of CPP-sponsored activities.

◊ Ghana is the only DFID target country in W. Africa, and rice is a relatively low priority crop, although production is increasing. Difficult to see how the CPP can effectively contribute to DFID's goals by focussing on rice production in Ghana given its high cost. Technologies developed through projects based in W.Africa may feed into other regions i.e. E.and central Africa, but communications within Africa are poor and efforts to understand how a new initiative coordinated by ASARECAwill address this issue should be investigated.

◊ DFID already has a number of good contacts with WARDA, and with NARs in Tanzania and Uganda. However, the development and strengthening of linkages between these partners could be improved to the benefit of all concerned. Collaboration between WARDA and IRRI is increasing.

◊ Existing links between WARDA and DFID need to be much more clearly and specifically acknowledged. Clear communication of programmes and activities is essential to the optimisation of impact, and the avoidance of duplication of effort. The document published by DFID giving descriptions of research which they currently fund in CG centres (Sept 1998) has been distributed by the CPP Programme Manager to project leaders. This shows that integrated management of RYMV and characterisation of blast genetic diversity and development of donors for durable blast resistance are the two major topics at WARDA which are funded with assistance from DFID. Stronger links with WARDA should be developed by the CPP project leaders.

◊ The lack of seed multiplication facilities represents a major bottle-neck in dissemination of improved seed, once released. Previous systems for seed multiplication have collapsed, often through lack of ongoing governmental and non-governmental support. WARDA intends to address this problem through community-based seed multiplication and contracting of local farmers, but the success of this approach will depend, at least in part, on the farming systems being employed in specific countries.

Table 1: Research activities to be carried out by WARDA member countries, into blast, brown spot and RYMV:

Research Activities	Blast	Brown spot	RYMV
Identification of key sites	Burkina Faso, Cameroon, Côte d'Ivoire, Ghana, Nigeria, Sierra Leone	Cameroon, Côte d'Ivoire, Nigeria, Sierra Leone	Burkina Faso, Nigeria, Côte d'Ivoire, Sierra Leone
Evaluation of economic importance	Burkina Faso, Cameroon, Côte d'Ivoire, Ghana, Nigeria, Sierra Leone	Ghana, Nigeria	Burkina Faso
Genetic diversity of disease population	Burkina Faso, Cameroon, Sierra Leone		
Epidemiology	Burkina Faso, Cameroon, Nigeria, Sierra Leone		Burkina Faso, Nigeria, Niger, Sierra Leone
*management of long-lasting resistance or **screening	*Burkina Faso, Cameroon, Sierra Leone, Senegal	**Nigeria, Sierra Leone	**Niger, Sierra Leone
Agricultural practices	Burkina Faso, Cameroon, Sierra Leone, niger,	Cameroon, Côte d'Ivoire, Ghana, Niger, Nigeria, Sierra	Sierra Leone

	Senegal	Leone, Senegal	
Chemical control	Burkina Faso, Cameroon, Niger, Sierra Leone, Senegal		
Biological control	Nigeria		

Table 2: Research activities into sheath blight (*Rhizoctonia solani*) bacterial leaf blight (*Xanthomonas campestris pv. Oryzae*) and leaf scald (*Gerlachia oryzae*):

Research activities	Sheath blight	Bacterial leaf blight	Leaf scald
*Identification of key sites **Monitoring	* Côte d'Ivoire, Ghana (*)(**) Nigeria		Nigeria (Dr. Singh)
Evaluation of economic importance	Nigeria	Niger	Nigeria (Dr. Singh)
Epidemiology		Niger	
Screening and varietal resistance	Ghana, Nigeria	Burkina Faso, Cameroon, Niger	Nigeria (Dr. Singh)
IPM components	Nigeria (chemotherapy, bio-control)	Niger (agricultural practices)	

Table 3: Crop Protection Constraints identified by workshop participants:

CONSTRAINT	CURRENT STATUS	EMERGING STATUS
Weeds	✓✓✓	✓↗
Blast	✓(work ongoing in Asia and other countries)	✓
RYMV	✓✓	✓✓✓
Nematodes	✓	✓✓
African Rice Gall Midge	✓	✓↗
Brown Spot / Seed Health	✓	✓✓
Stem Borers	✓	✓?
Birds	✓✓✓	✓✓✓
Rodents	✓✓ - current initiatives ?	✓✓✓
Termites	✓	?

✓ : Some economic significance
✓✓ : Serious constraint
✓↗ : Serious constraint and likely to increase
✓✓✓ : Severe constraint

Factors which may adversely affect efforts to address the above constraints:
- Uptake of new technologies
- Integrated crop management - development of ICM strategies with and adoption by small-scale farmers
- Lack of information / monitoring of changing systems

Table 4: Current partnerships addressing specific constraints:

Constraint	Institutions
Weeds	WARDA, IRRI & partners, ORSTOM, NARS
Blast	World-wide efforts ongoing, but those specifically relevant to Africa include: Kansas, Davis, Purdue and Cornell Universities (USA), IRRI, WARDA, NARS, CABI, HRI, NRI
RYMV	WARDA, DFID (JIC under PSRP, NRI under CPP), ORSTOM, ILTAB, IRRI (Madagascar), NARS
Nematodes	Leeds University / WARDA / CABI, ORSTOM, IRRI, NARS
Gall Midge	Asian Gall Midge: IRRI, ECGEB, Delhi and others African Gall Midge: WARDA / CABI, NARS
Seed health	IRRI / CABI / BRRI, WARDA

7. **Primary areas and approaches for research:**
   - Integrated crop management - adaptive research, directed primarily at rainfed lowland systems
   - Capacity building in biotechnology and collaboration

8. **Plant Science Research Programme meeting**

   The CPP workshop delegates attended the Plant Science Research Programme meeting on rice biotechnology at Leicester between the 9th and 10th December.

   Drs F.Kimmins and J. Lenne represented CPP management and FMK was requested to provide information on CPP priorities together with suggestions for linkages between the two RNRRS programmes through the existing and future projects. Other CPP delegates attended as observers.

9. **Current projects**
   The programme for the PSRP meeting is given in Appendix 2 with the titles of current and future PSRP projects and project leaders.
   The 'current' PSRP projects which complement CPP projects are:
   - R6948   Rice transformation (High Potential) P.Vain, John Innes Centre (JIC).
     Start 1/09/97 End 31/08/2000
   - R6394   Assessing transgenic lines for rice resistance to tungro virus (High Potential) R.Hull JIC.
     Start 1/11/95 End 31/10/98
   - R6355   Transgenic resistance to rice yellow mottle virus (Semi-Arid) (Y Pinto, Sainsbury Laboratory, JIC).
     Start 1/7/95 End 30/6/98
   - R6453   Transgenic crop resistance in upland and lowland rice to nematodes (Semi-Arid) H Atkinson, Leeds University.
     Start 1/1/96 End 31/12/98
   - R6442   Cassava genetic engineering for virus resistance (Forest-Agriculture) H Luong IACR Rothamsted and JIC.
     Start 1/12/95 End 30/11/98

   Many of these projects have finished this year and are under review prior to development of a second phase or cessation of funding. R6394 (Assessing transgenic lines for rice resistance to tungro virus) and R6442 (Cassava genetic engineering for virus resistance) are unlikely to be

continued under the PSRP. It appeared from the presentations that there had been insufficent formal interaction between the CPP and PSRP projects, although it was clear that they would have benefited from better communication and liaison between the two programmes.

10.   Future PSRP projects
PSRP projects which will be developed in the future include:

    RYMV resistance in rice (focusing on field testing of transgenic lines in Cote D'Ivoire and mapping of molecular markers in interspecific hybrids of *O.sativa* and *O.glaberrima.* (Semi-Arid, Y Pinto, Sainsbury Laboratory, JIC and WARDA).

- Full and durable resistance in rice and potatoes to nematodes (Semi-Arid , Upland rice; High Potential, irrigated rice and Hillsides, Potato. H Atkinson, Leeds, WARDA, IRRI and PROINPA, Bolivia). Continuation of previous project to develop constructs which will deliver combinations and anti-nematode gene products under control of root-specific promoters. 1/1/99-31/12/01.

- Banana and plaintain transformation. P.Vain (JIC and African NARS?).

11.   PSRP and CPP linkages
Project leaders on the CPP and PSRP projects have developed informal linkages and exchanged materials and information during the course of the projects. For example, isolates of RYMV collected in Tanzania on the CPP project were given to the PSRP project so that i) the sequence of ORF2 could be compared to the sequences from other isolates and ii) they could be used against the transgenic rice plants under glasshouse conditions at JIC. This informal collaboration will continue during the next phase.

Little exchange of information had taken place between the CPP nematode projects and the PSRP project. In part this was due to the lack of a CPP upland rice project, but opportunities for interactions were available as a nematologist placed at WARDA had been funded under the previous IPM strategy and RNRRS CPP (1995-1998).   Dr Atkinson felt that future interaction with 'field' scientists involved in CPP rice nematology and potato IPM projects would provide the skills missing from his project and provide essential background information on pest interactions, cropping systems and farmer perceptions of crop production constraints.
Action point: Dr Waller to contact Drs Snape and Witcombe about CPP potato project in Bolivia and development of project linkages with the PRSP project.

Similarly, the new PRSP work on banana transformation would benefit from linkages with the CPP project in Uganda which has looked at nematode variability (R6583). It will contain useful information about varieties preferred by farmers and farmers perceptions of nematode damage. Some interaction between Dr Gowen (Reading University) and the PSRP had occurred but stronger linkages are needed to ensure that the information from the CPP projects are made available to the PSRP project.
Action point. Dr Waller to contact Drs Snape and Witcombe with CPP nematology inputs on banana in Uganda.

12.   General conclusions
It was generally concluded that the joint meeting with CPP and PSRP project staff had been productive, but there was definite need to significantly enhance cross-programme communication. It was suggested that the programme managers should meet and discuss these linkages, but the PSRP PAC adviser from DFID, pointed out that the managers already met at their own annual meeting and at the NR advisers conference. Another suggestion was that project leaders should develop informal linkages through their projects and develop exchange visits in selected cases. i.e. RYMV, potato and banana project leaders from the respective programmes. The CPP PSLs could fund this as a dissemination activity where a visit was

merited. Cross-programme meetings such as the rice workshops encourage linkages but are expensive and cannot be held every year. A CPP Rice in Asia workshop, however, is due to be held in Bangladesh (early April). Dr Witcombe should be invited to select a participant from his programme to attend the meeting (cost to be borne by PSRP).

Action point. PSLs to discuss potential linkages. Dr Kimmins and or Eden-Green to contact Dr Witcombe about outcome of CPP discussions and development of better communication pathways through CPP workshops and projects.

Frances Kimmins & Helen Turner
January, 1999

ANNEX I: Workshop Participants

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RICE LEAF SCALD: PATHOGEN BIOLOGY AND DIVERSITY

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1. Introduction

Rice leaf scald is caused by the fungal pathogen *Monographella albescens* (Thüm) Parkinson, Sivanesan and Booth (anamorph *Gerlachia oryzae* (Hashioka and Yokogi) W. Gams). It is a disease that can be found throughout virtually all of the rice growing regions of the world (International Mycological Institute, 1996). Under favourable climatic and cultural conditions, *M. albescens* can pose a serious threat to rice production and is often ranked in the first three or four fungal constraints for a region, along with blast (*Magnaporthe grisea*), sheath blight (*Rhizoctonia* spp.) and brown spot (*Helminthosporium oryzae*) (Jones *et al.*, 1991; Alam *et al.*, 1985; Cuevas-Perez & Gaona, 1988; Kempf, 1983; Prabhu & Sitarama-Prabhu, 1989). Incidence of infection in an area can reach high levels under favourable conditions, resulting in significant loss of yield, such as the 23.4% yield reduction reported in India by Srinivasan (1981).

Much of the literature relating to this disease comprises first reports of the pathogen's presence in a given region, or revisions of the taxonomy of the anamorph and teleomorph. Variations in symptomology and host tissues affected have given rise to a variety of common names for the disease, for example leaf scald, brown leaf blight, leaf tip blight, leaf tip drying (Ou, 1985) and brown leaf spot (Naito, 1982). Ou (1985) produced a succinct overview of the taxonomy of the pathogen, while Parkinson *et al.* (1981) have provided good evidence for the current classification, with *Monographella albescens* superseding *Metasphaeria albescens* (Thum) for the teleomorph, and *Gerlachia oryzae* superseding *Rhynchosporium oryzae* (Hashioka & Yokogi) for the anamorph.

Despite the wide geographical distribution of *M. albescens*, this pathogen has been the subject of remarkably little research, being overshadowed by the more damaging rice blast pathogen *Magnaporthe grisea* (anamorph *Pyricularia oryzae*). However, as blast-resistant rice varieties have been developed and introduced into general use around the world, it is worth bearing in mind that leaf scald is a near-ubiquitous disease that can become a severe problem when favourable conditions are prevalent. In light of this, some attention should be devoted to the biology of the causal agent and particularly to

the levels of genetic and phenotypic variability within and between populations. Current understanding of these aspects is therefore discussed below.

2. Morphology and Culture

M. albescens is a uninucleate, filamentous fungus (Hyponectriaceae, Ascomycota) which is most often observed causing characteristic light-and-dark banded brown lesions which extend down the leaf from the tip or inwards from leaf margins, drying the leaf tissue as the lesion develops (Figure 1, arrowhead). Scald infection can occur in conjunction with other diseases, such as blast (Figure 1, arrow). *M. albescens* has also been shown to infect the leaf sheath (Singh, 1989), seeds (Wu, 1994; Thomas, 1984; Mia *et al.*, 1985), spikelets and panicles (Ribeiro, 1983).

Figure 1: Rice leaves showing typical leaf scald symptoms. Note how the lesion, spreading down from the leaf tip, shows a distinctive banding pattern (arrowhead). These leaves also show signs of infection by the rice blast pathogen *Magnaporthe grisea* (arrow).

The pathogen forms falcate, predominantly bitunicate conidia on the leaf surface (Figure 2); the conidiogenous structures often emerge from stomata. The conidia are 9-14 μm long by 3-4.5 μm wide (Ou *et al.*, 1978). Perithecia, containing colourless paraphyses and sets of eight ascospores per ascus, develop within the mesophyll layer of infected leaves.

Figure 2: Formation of bitunicate conidia (arrows) at the leaf surface. This figure shows fungal material on the surface of a rice leave. The infected tissue was cleared in methanol, stained with fluorescent brightener and observed under UV illumination. Scale bar represents 20μm.

M. albescens can be cultured on potato dextrose agar (PDA) where it will often produce an abundant pale pink or cream mycelium (Parkinson, 1980). Conidia form on ageing cultures in orange-pink gelatinous patches, particularly if the medium is supplemented with an aqueous extract of rice leaves. However, prolonged maintenance *in vitro* results in attenuation of the isolates' pathogenicity and sporulation capacity (Turner and Black, 1996). Crill *et al.* (1981) succeeded in inducing perithecial formation in cultured isolates, by crossing isolates on rice leaf sheaths placed on 1.7% agar containing Sach's medium, but were unable to determine whether the pathogen was homothallic or heterothallic. Production of perithecia outside host tissues has not been achieved.

3. Sources of Infection

M. albescens is primarily a foliar pathogen, usually invading host tissues from the leaf tips and margins. There is some debate as to the major sources of inoculum in the field. A number of papers have been published describing the isolation of the pathogen from rice seeds and implicating seed transmission as an important mechanism (Mia *et al.*, 1989; Mia and Safeeullah, 1986). However, Singh and Gupta (1986) reported minimal incidence of *M. albescens* on seeds collected from heavily infected rice fields and found that plants raised from infected seeds remained disease-free. Clearly this is an important point which requires further clarification if effective disease control, by seed-treatment or by improved agronomic practices, is to be developed.

The possibility that viable pathogen inoculum persists in crop detritus has been examined. Singh and Gupta (1986) found that viable inoculum persisted for no more than a few months on leaves buried in the soil, although this period would be long enough to provide inoculum where rice is cropped on a bimodal basis. However, scald is more commonly a problem in non-irrigated cropping systems where rice is grown on an annual basis. Thomas (1984) reported that the pathogen did not survive the dry season (November - May) under field conditions, but survived in harvested seeds, stored at 26-28°C, for over a year. At NRI, we have found that viable isolates can be obtained from dried rice leaves at least a year after collection from the field (Turner and Black: unpublished data) although some loss of virulence may have occurred. Also, laboratory conditions for leaf preservation were far more favourable than those likely to prevail in the field.

Alternate hosts are another potential source of inoculum for this disease. Several host species have been identified amongst graminaceous weeds commonly found around the margins of rice fields, for example the grass *Echinocloa crus-galli* (Singh and Gupta, 1981); *Chloris* sp*., Eleusine* sp*., Melinis* sp*., Rhynchelitrum* sp*., Setaria* sp. and various *Oryza* spp. (Prabhu and Bedendo, 1982); *Oryza, perennis, Echinocloa crus-galli* and *Brachiaria mutica* (Das, 1994). The transmissibility of *M. albescens* between these potential hosts and cultivated rice would bear further investigation, as would the phenotypic and genetic similarities between isolates found on rice and non-rice hosts.

4. The Infection Process

Conidia are transmitted by water-splash and germinate on the host surface. Singh and Gupta (1983) have shown that rice leaf exudates and extracts stimulate conidial germination and germ-tube growth. Interestingly, this stimulation was found to be more pronounced with extracts from susceptible as opposed to resistant rice cultivars.

The germ-tubes that emerge from the conidia often anastomose (Naito, 1982; Ou, 1985) and Naito (1982) reported that infection by anastomosis complexes was significantly more likely to be successful than infection originating from solitary conidia. Koiso *et al.* (1986) demonstrated that some host-derived carbohydrates stimulate anastomosis in *M. albescens*.

Figure 3: Stages of rice leaf infection by *M. albescens*. (a) Hyphae at the leading edge of a lesion show a preferred orientation that parallels the direction (arrowed) of the longitudinal ridges of the leaf surface. Scale bar represents 40μm. (b) Behind the leading edge of the mycelium, side-branches develop. Where stomata are encountered appressoria (arrow) are formed and the stomatal aperture penetrated. Scale bar represents 40μm. (c) Deeper within the lesion, proliferation of hyphal branching and anastomosis result in a complex hyphal mat over the leaf surface. Scale bar represents 20μm. (d) Direct penetration of the leaf surface, via the bulliform cells (B). Surface and internal hyphae indicated by arrows. Scale bar represents 10μm. (a) to (c) show surface fungal material on methanol-cleared rice leaves, stained with fluorescent brightener and observed under UV illumination. (d) shows a transverse section of a methanol-cleared, infected leaf stained with 0.05% toluidine blue.

Once the conidia have germinated, the hyphae start to spread over the surface of the rice leaf. We have found that, at the leading edge of the mycelium, the hyphae often run parallel to the longitudinal contours of the leaf surface (Figure 3a). Lateral branches develop from these exploratory hyphae. Where stomata are encountered, the hyphae form swollen, appressorial infection structures and penetrate the host through the stomatal apertures (Figure 3b). Behind the leading edge of the lesion, the mycelium continues to develop, forming a complex mass of inter-connected hyphae in which no preferred orientation can be discerned (Figure 3c).

It should be noted that, although stomatal penetration is the most frequent method of infection, *M. albescens* is also capable of direct penetration of host tissues. Naito (1982) demonstrated that this is particularly the case for tissues lacking the heavy deposits of cuticular waxes common to leaf surfaces. Our own observations have confirmed that direct penetration can occur, even into leaves with their heavily-waxed cuticles, notably via the bulliform cells (Figure 3d), although stomatal penetration was still the predominant method of entry.

Figure 4: Transverse section of an infected rice leaf, showing the intercellular ramification of *M. albescens* hyphae (arrows) between the mesophyll cells (M). The epidermis (E) is visible at the top right. Infected leaf material was cleared in methanol, sectioned on a Bright Cryostat microtome and stained with 0.05% toluidine blue. Scale bar represents 5µm.

Once the host leaf has been penetrated, the hyphae ramify between the mesophyll cells (Figure 4). This penetration and intercellular ramification begins in the green region of the leaf ahead of the expanding necrotic zone. Some penetration of the hosts mesophyll cells occurs (Naito, 1982) as the intercellular network develops and the leaf tissues begin to die off. Phenolic compounds are deposited and it becomes harder to distinguish the fungal material present in this necrotic zone. Viable hyphae do persist, however, for it is in the necrotic zone that perithecia are formed (Figure 5).

Figure 5: Longitudinal section of the necrotic zone of an *M. albescens* lesion, showing a perithecium (P) and asci (arrows). The infected leaf originated from Nigeria. The leaf material was cleared in methanol, sectioned on a Bright Cryostat microtome and stained with 0.05% toluidine blue. Scale bar represents 20μm.

M. albescens, grown in liquid medium (potato dextrose broth, supplemented with aqueous rice extract), produces one or more compounds that are toxic to rice plants, inducing chlorosis, wilting and necrosis (Maji and Gupta, 1986; Singh and Gupta, 1983). This toxicity was found to be specific to rice plants, and may comprise an important mechanism contributing to the successful invasion of the host by the pathogen. Research within our own laboratory has demonstrated that conidia held in aqueous suspension for 48 hours can also release one or more host-specific toxic components into the water (Turner & Black, 1996) and that these components are dependent on light for their activity (Noor Azman, 1994). Elucidation of the conditions for maximum toxin production and of the nature of the toxin(s), however, was hampered by loss of virulence in the pathogen, and by attenuation of toxin production, during maintenance of the pathogen in culture.

5. Host Resistance

In a number of regions where leaf scald presents a significant constraint to rice production, screening of cultivars for resistance has been introduced. For the most part, this has been done through field-trials, usually using the International Rice Research Institute (IRRI) standard evaluation system to determine relative disease severity. The proportion of tested rice lines identified as showing high levels of resistance, according to these standards, has often been disappointingly low. For example, only 5 selections out of 437 showed high resistance, with another 33 selections showing moderate resistance in India (Das, 1976). More recently, Saifulla (1993a) found only one scald-resistant entry out of 490 accessions tested at Karnal. Verma and Singh (1982) found

24 resistant cultivars out of 589 cultivars tested in 1980 but only 2 out of 152 cultivars tested in 1981. Under lowland conditions in Indonesia, Rahamma (1986) found 10 out of 100 cultivars showing good resistance. Saifulla (1993b) also reported an accession that showed relatively high levels of resistance to both scald and blast.

Ribeiro (1983) reported that the incidence of severe scald disease episodes has increased since the introduction of semi-dwarf rice varieties to Brazil. This is a worrying trend, given that improved introductions are commonly of semi-dwarf stock. However, these data also suggest that the traditional varieties may well prove to be a valuable source of scald-resistance traits that could be of use in future breeding programs, especially if these traits can be clearly identified.

Thus far, no specific mechanisms for host resistance have been identified, nor any genetic basis for resistance, making it difficult for breeding programs to fix resistance traits into agronomically useful rice lines. Generally speaking, even 'resistant' cultivars can be infected by *M. albescens*, but will suffer only limited lesion development and little if any loss of yield. Bonman *et al.* (1990) tested 288 rice accessions for resistance to *M. albescens*, using an *in vitro* method and found that, rather than distinct groupings of resistant, tolerant and susceptible accessions, there was a continuum from low to high levels of resistance, with no accessions showing complete resistance to the pathogen. This suggests that the interactions between *M. albescens* and rice plants is not dominated by major-gene resistance, unlike the resistance traits identified in barley cultivars against barley leaf scald, *Rhynchosporium secalis* (Graner and Tekauz, 1996; Schweizer *et al.*, 1995; Abbott *et al.*, 1995). Clearly, more work needs to be done if the relationship between *M. albescens* and its host is to be understood and this understanding used to improve the disease resistance of rice cultivars developed in the future.

It has been noted on several occasions that the application of nitrogenous fertiliser, or growth of rice on nitrogen-rich soils, results in a general increase in the susceptibility of rice plants to leaf scald (Das *et al.*, 1994; Misra and Mathur, 1982; Mondal *et al.*, 1986; Singh and Gupta, 1983). Interestingly, Winslow (1992) reported that supplementing the levels of soluble silicon available to *indica* rice plants in Nigeria significantly reduced the severity of leaf scald disease. Improved resistance may have been conferred through increased deposition of silicon at the leaf surface. However, Winslow noted that upland *japonica* types, which had up to 100% higher levels of silicon in their leaves than the lowland *indica* types, proved to be equally susceptible to leaf scald, which argues against this hypothesis. Also, the propensity of *M. albescens* for entry into the leaf via stomata, rather than by direct penetration of the epidermis as effected by the blast pathogen, would seem to provide a ready method for bypassing such a structural defence.

6. Biological and Molecular Diversity of the Pathogen

The phenotypic and genotypic variability of *M. albescens* are areas that have been almost completely neglected to date. Below we have summarised the information currently available and outlined some of the work presently in progress to improve on this situation.

6.1 CULTURAL DIVERSITY

Singh and Gupta (1985) published an analysis of variation among six isolates of the pathogen obtained from various regions of India, noting that these isolates showed differences in total conidial biomass production, when grown in liquid culture. They also conducted some assessment of the isolates' pathogenicity, by spraying seedlings of a susceptible cultivar (Jaya) and a resistant cultivar (Dular) with conidial suspensions. Differences in pathogenicity were found to be more pronounced on the susceptible Jaya seedlings.

Parkinson (1980) described four main groups of fungal morphology for *M. albescens* isolates grown in culture, distinguished by the abundance of aerial hyphae and conidial masses produced and the intensity of the mycelial pigmentation. Fungal morphology *in vitro* should, however, be treated with caution. We have observed significant changes in the production of aerial hyphae and conidial masses by isolates held in culture over a period of several months. No relationship has been established between morphological type of an isolate in culture and its pathogenicity, but we have observed that isolates of *M. albescens* held in culture do suffer attenuation of pathogenicity over a period of several months which can be associated with a loss of sporulation capacity.

6.2. PATHOGENIC DIVERSITY

Laboratory experiments designed to assess the resistance of various cultivars to leaf scald disease have almost invariably utilised only one or two isolates of the pathogen. For example, Bonman *et al.* (1990) used an *in vitro* method, as well as whole-plant inoculation, to determine the relative susceptibility of 288 rice accessions to *M. albescens*. However, only two isolates, both from the Philippines, were used to challenge the host plants. Even then, some variation in the isolates' pathogenicity towards the various rice accessions was noted and led the authors to suggest that this was due to pathogenic specialisation. The authors also speculated that such specialisation may account for disparities within the literature reporting on the relative susceptibility of certain cultivars to leaf scald.

In collaboration with the West African Rice Development Association (WARDA), we have begun to assess the pathogenic variability of *M. albescens* isolates. *In vitro* analyses, following the detached leaf protocol described by Bonman *et al.* (1990), are being used to investigate the pathogenicity of selected West African isolates of *M. albescens* against a range of rice cultivars. Segments of fully expanded leaves from two month old rice plants are placed on 2% water agar plates, and inoculated with 3mm-diameter mycelial plugs taken from the margins of *M. albescens* cultures grown on

PDA (Oxoid). The length of developing lesions is determined at 3 days after inoculation. Results of one such experiment, with 12 replicates per isolate/cultivar combination, are shown in Figure 6.
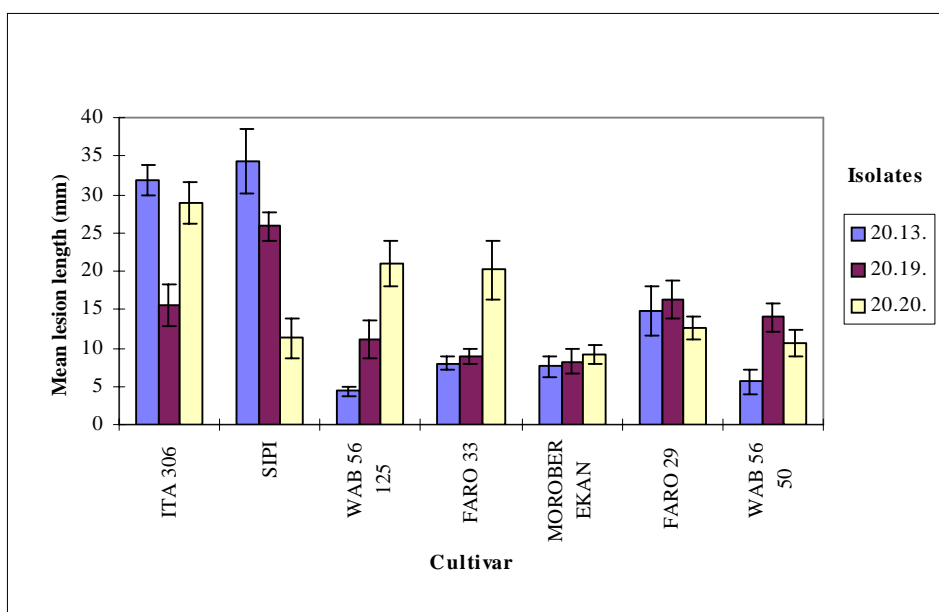

Figure 6: Mean lesion length (in mm +/- 95% confidence limits) at three days after inoculation of detached rice leaves with plugs of *M. albescens*, following the protocol of Bonman *et al.* (1990). Isolates 20.13, 20.19 and 20.20 were isolated from leaf material collected from a WARDA trial site in the Ivory Coast, and inoculated onto the rice varieties indicated right. Control replicates, where rice leaves were inoculated with PDA only, did not form lesions.

As can be seen in figure 6, the pattern of virulence for each isolate towards the various rice cultivars is distinctive. For example, while both isolates 20.13 and 20.20 do well on cultivar ITA 306, and isolate 20.19 shows only intermediate lesion development, this situation changes when cultivar SIPI is used: both 20.13 and 20.19 do well and it is isolate 20.20 that shows limited lesion development. Characteristic patterns of pathogenicity, even within this small set of isolates, are becoming apparent. These findings go some way towards supporting the suggestion of pathogenic specialisation made by earlier workers (Singh & Gupta, 1985; Bonman *et al.,* 1990). The potential existence of *M. albescens* populations geographically distinguishable by pathogenic specialisation would have significant implications for local and international breeding programs wishing to incorporate scald-resistance traits into rice germplasm.

In vivo analyses are also in progress at NRI, in parallel with the detached-leaf protocol outlined above. Clip-inoculation of seedlings at the 5-leaf stage is carried out by cutting the tip off fully-expanded rice leaves with mycelium-coated scissors. The inoculated plants are then enclosed in a plastic bag, in order to maintain the high level

of humidity required for consistent levels of infection. At 6 days after inoculation, the leaf tips are collected into methanol for clearing. After 24 hours, the leaf tips are stained with fluorescent brightener (Sigma Chemical Co.) and viewed under ultra-violet illumination with an Olympus BH-2 microscope. Where infection has been successful, the pathogen can be clearly seen growing over both abaxial and adaxial leaf surfaces. Although more labour-intensive, this method does more closely simulate field conditions, where whole-plant responses may be important to the plants' resistance to, or tolerance of, leaf scald infection.

6.3. GENETIC DIVERSITY

Given the limited research that has been directed towards phenotypic variability of *M. albescens*, it is not surprising that the genetic variability of this pathogen on different continents, or within different agro-ecological systems in the same country, remains unknown. However, we have now begun studies, in conjunction with the phenotypic work outlined above, on genetic variability among and within *M. albescens* populations from S. E. Asia, West Africa and South America. Isolates of *M. albescens* obtained from diseased leaf material collected from these regions, with the assistance of IRRI, WARDA and the Centro Internationale de Agricultura Tropicale (CIAT) respectively, were grown in potato dextrose broth and the DNA extracted according to a protocol adapted from that of Lodhi *et al.* (1994). A portion of the ribosomal DNA (rDNA) was then amplified by polymerase chain reaction (PCR) using the conserved-region primers ITS-1 and R635. These primers amplify the variable internal transcribed regions 1 and 2, the 5.8s gene and part of the 28s gene. Aliquots of the amplified product were then digested with selected nucleases, including Hinf I, Hae III, Rsa I, Hpa II, Taq I and Hha I. The digestion fragments were separated on a 2% agarose gel, using 0.5x TBE buffer and the restriction fragment length patterns were visualised on a UV trans-illuminator after staining the gel with ethidium bromide.

The region of rDNA analysed showed little variability. Isolates could be divided into two groups only, based on the restriction digest patterns from digestion with Hha I (Figure 7). Both groups were represented in isolate collections from each of the countries of origin examined. Sequencing of PCR product from this region in selected isolates has shown that the two Hha I-derived AFLP groupings result from a single base variation within an Hha I restriction site in the second internal transcribed spacer region (ITS 2). Analysis of the intergenic spacer (IGS) region of the *M. albescens* genome is in progress at NRI and a greater degree of variability has already begun to emerge in this region.

Kb

--- 0.5

--- 0.4

--- 0.3

--- 0.2

--- 0.15

Figure 7: DNA amplified from the ITS region of the genome, using the polymerase chain reaction, was digested with Hha I. All isolates in this figure were isolated from material collected in the Ivory Coast. Isolates are as follows: Lane 1 = F37-3, lane 2 = DP2, lane 3 = MOR1, lane 4 = MOR2, lane 5 = SHIN2, lane 6 = F37-3b, lane 7 = W5650-2, lane 8 = DY3, lane 9 = IR5-3, lane 10 = IDSA6-1, lane 11 = IDSA6-2, lane 12 = MOR3, lane 13 = M22-1, lane 14 = NP2, lane 15 = molecular weight standard (Kb ladder, Gibco BRL).

In time, we should be able to determine any correlation with geographic source or pathogenic virulence of the isolates. Information on the genetic diversity of this pathogen will provide valuable clues to the history and biology of *M. albescens*.

7. Summary

M. albescens, the causal agent of the rice leaf scald disease, is an agronomically significant pathogen in many rice-growing regions of the world. The infection process has been described by various authors, but many aspects of this process are still not clearly understood. For example, the contribution of host-specific toxins to the pathogen's success before and after penetration of the host is not known. Infected seeds, detritus from previous crops and alternate hosts have each been suggested as probable sources of primary inoculum under field conditions, but no consensus has been reached on their relative importance generally, or even under specific cropping systems. Field trials have indicated that some rice varieties have useful levels of resistance or tolerance to local populations of *M. albescens*, but the mechanisms of resistance have not been identified. The existence of pathogenic specialisation among *M. albescens* populations and even of physiological races has been suggested, but remains unconfirmed. Essentially, *M. albescens* is a little-studied pathogen, but work is in progress to better understand its epidemiology and genetic variability, with a view to developing more effective control of rice leaf scald.

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