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Pathogenic variability in *Mycosphaerella fijiensis* and its implications for disease resistant bananas and plantains

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ABSTRACT

For resistance to black leaf streak (black Sigatoka) (*Mycosphaerella fijiensis*), breeders have concentrated on resistance that is simply inherited and can be readily recovered in the diploid improvement phase of the breeding programme. The principal source has been the diploid clone Calcutta 4 (*Musa acuminata* ssp. *burmannicoides*). Host inoculation studies have revealed considerable pathogenic variability within *Mycosphaerella fijiensis* Morelet particularly in the Papua New Guinea/South Pacific Region. It has also been shown that resistance in some genotypes (e.g. Paka, Yangambi km5) is strain-specific and that there are strains of the pathogen capable of overcoming it. Strains capable of infecting juvenile plants of Calcutta 4 have been found in Papua New Guinea and the South Pacific. There is a high risk associated with the continued reliance on a narrow genetic base for resistance breeding on a global scale. Priority should be given to identifying alternative sources of resistance, determining numbers of genes conferring resistance, screening germplasm with strains of known virulence, and utilising general (partial) resistance wherever possible. Resistance to *Mycosphaerella eumusae* should also be included as an additional target in breeding programmes.

Key words: Banana; black leaf streak; disease resistance; *Mycosphaerella fijiensis*; pathogenic variability

RÉSUMÉ

Pour la résistance à la maladie des raies noires (Sigatoka noir) des bananiers et des plantains (*Mycosphaerella fijiensis* Morelet), les améliorateurs se sont concentrés sur la résistance a héritabilité simple et qui peut être facilement récupérée pendant la phase d'amélioration du diploide. La principale source de résistance a été le diploide *Musa acuminata* ssp. *burmannicoides*, clone Calcutta 4. Des inoculations sur plantes hôtes utilisant des souches choisies de *M. fijiensis* ont montrées une très grande variation du pouvoir pathogène en particulier chez les souches de Papua Nouvelle Guinée et chez celles de la région Pacifique Sud. Il a été montré que la résistance chez certains gènotypes (e.g. Paka, Yangambi km5) est souche-spécifique et qu'il y a des souches qui sont capables de la surmonter. Des souches capables de causer la maladie sur jeunes plantes de Calcutta 4 ont été trouvées en Papua Nouvelle Guinée et dans le Pacifique Sud. Il y a un gros risque associé avec la dépendance continuelle à un echelon global d'une résistance à base étroite telle que Calcutta 4. Prioritée devrait être donnée à l'identification de nouvelles sources de résistance, à la déterminiation du nombre de gènes qui codent pour cette résistance, à la sélection de gènotypes utilisant des souches de virulence déterminée pour distinguer entre résistance qui est souche spécifique de celle qui est générale, à l' utilisation la résistance multigène (partielle) dans les programmes de selection. La résistance à *Mycosphaerella eumusae* devrait aussi être inclus comme objectif supplémentaire dans les programmes de sélection.

INTRODUCTION

The global spread of black leaf streak (black Sigatoka) (*Mycosphaerella fijiensis* Morelet) following its first detection in a commercial banana plantation in Fiji in 1964 (Rhodes 1964) represents one of the classic plant disease epidemics of modern times. Despite its impact on large-scale commercial production, it was the effect on subsistence and small-scale commercial growers in Central America and Africa that focussed world attention on the disease. The plantain types of banana (AAB) were all highly susceptible to black leaf streak and the 'new disease' threatened the food supply of millions of people for whom banana was a staple food crop. Immediate priority was given to the breeding of resistant cultivars to replace the highly susceptible cooking varieties.

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The breeding programmes in Honduras and West Africa have been highly successful, with a number of disease-resistant cooking bananas being released to farmers over the past decade. All of these programmes have relied heavily on the diploid clone Calcutta 4 (*Musa acuminata* ssp. *burmannicoides*) as a source of resistance. As a result, the majority of varieties being released carry the same resistance. There is the potential for the failure of those varieties should a strain of the fungus emerge that can overcome that resistance. This paper presents an overview of pathogenic variability in *M. fijiensis* and its implications in the search for alternative sources of resistance.

MECHANISMS OF RESISTANCE

The resistance expressed by plants to invading pathogens falls into two broad categories:

1. *Strain-specific resistance* (also commonly known as '*specific resistance*', '*vertical resistance*', '*major gene resistance*' and '*qualitative resistance*'). A cultivar possessing strain-specific resistance will be typically resistant to some strains of a pathogen but not to others. The resistance is normally controlled by a single 'resistance' gene in the host that is able to recognise, and respond to, strains of the pathogen that carry a corresponding 'avirulence' gene. Penetration by the fungus leads to the rapid death of the invaded cell and often a number of adjacent cells. The invading fungus becomes isolated in an island of dead tissue and can progress no further. This response is known as a 'hypersensitive' reaction. A single mutation in the pathogen (resulting in the loss of the avirulence gene) could produce a strain of the fungus that is unrecognisable to the previously resistant host plant and thus can successfully infect. At that stage the resistance is said to have 'broken down'.
2. *Strain-non-specific resistance* (also commonly referred to as '*general resistance*', '*horizontal resistance*', '*minor gene resistance*', '*quantitative resistance*' or '*partial resistance*'). With resistance of this kind, infection by the pathogen will stimulate a range of resistance responses that either suppress the development of the pathogen, or limit its effect on the host. The various host responses are controlled by different genes and the effects are normally additive. A single mutation in the pathogen will have little effect on the overall disease resistance in the host. If one resistance mechanism is overcome, a range of others will continue to inhibit the fungus. For that reason, strain-non-specific (general) resistance is considered to be more durable than strain-specific resistance.

BREEDING FOR RESISTANCE

Strain-specific resistance has been the preferred form of resistance for plant breeders of many types of food crops. It can readily be handled in pedigree breeding programmes or, in many cases, simply introgressed into existing varieties by a process of backcrossing and selection. By contrast, the breeding strategy for general resistance involves the progressive accumulation of minor resistance genes in good agronomic types by a programme of recurrent mass selection. Such a strategy is dependent on the ability to perform large numbers of crosses between parents and to select a population of superior progeny (agronomic quality/disease resistance) for the following cycle of intercrossing.

Conventional breeding of bananas utilises the low levels of female fertility in some triploid clones, and agronomically improved (including disease resistance) fertile diploids as males (Rowe 2000). As a result, banana improvement is largely restricted to a pedigree breeding strategy utilising simply inherited (and possibly less durable) sources of disease resistance. Because the resultant varieties are genetically 'fixed', when released globally they present ongoing selection pressure for virulence within local populations of the pathogen.

PATHOGENIC VARIABILITY IN *M. FIJIENSIS*

Molecular analyses (Carlier et al. 1994, 1996; Neu et al. 1999) have revealed extensive polymorphism in populations of *M. fijiensis* particularly in recognised centres of diversity of *Musa* such as South East Asia, Philippines and Papua New Guinea. Such analyses do not, however, relate directly to biological attributes of the organism, and infer only the likelihood of pathogenic diversity in any particular population.

To date, only one detailed study of pathogenic diversity in the organism using host plant reactions has been carried out (Fullerton & Olsen 1995).

Strains of the pathogen from different localities were inoculated to a range of *Musa* genotypes under standard conditions, and the reaction of each genotype/strain combination recorded. A selection of 54 strains of *M. fijiensis* were used for the study, mostly from the Pacific Islands and Papua New Guinea, but including representative isolates from Central America and Africa. The host genotypes were a "standard set" of varieties selected to provide a range of disease reactions.

Tests were made on juvenile plants approximately 6 weeks after growing-out from tissue culture. A suspension of conidia was applied to the abaxial surface of the two youngest, fully expanded leaves, allowed to dry for 1-2 hours then incubated at 25-28∞C under near-saturated conditions for 8 days. After the incubation period, plants were held at 25-28∞C for symptoms to develop. Leaves were inspected every 5 days, and the most advanced stage of symptom development and the time taken to reach that stage were recorded. From that data a series of grades of pathogenicity ranging from 1 (highly resistant host reaction) to 5 (highly susceptible host reaction) was developed and single rating then applied to each host/strain interaction.

Full details of *Musa* genotypes used, methods, and host responses of all strains tested are to be found in Fullerton & Olsen (1995). A representative selection of results of host/pathogen interactions is shown in Table 1. The principal findings were:

1. Different strains of the pathogen had different combinations of virulence.
2. Some hosts e.g. Grande Naine and SF215 were susceptible to all strains. Others e.g. T8, Calcutta and Pahang were susceptible to some strains but not to others. Only one, Tuu Gia, was consistently resistant although it expressed a moderately susceptible reaction to some strains.
3. The resistance expressed by some of the genotypes (e.g. Paka, Yangambi km5, Calcutta 4) was strain-specific and there were strains capable of overcoming it.
4. Virulence on Paka (an AA diploid of East African origin) and T8 (an AAAA Highgate x Paka hybrid) is widespread in the Pacific Islands and Papua New Guinea.
5. Resistance in Paka and Yangambi km5 may be conferred by the same gene.

Collectively, strains of *M. fijiensis* from the Papua New Guinea/South Pacific area expressed virulence on all genotypes used in the study except Tuu Gia. Many of the strains from the Pacific region carried a broad spectrum of virulence. The few strains tested from Central America, and Nigeria (both areas of relatively recent introduction) had a relatively limited spectrum of virulence.

The reaction observed in Calcutta 4 is of particular interest. Virulence on juvenile plants was commonly found in stains from Papua New Guinea and the Pacific Islands. However there is, to date, no record of Calcutta being diseased in the field, suggesting that different mechanisms of resistance are operating in the adult plant. Possible differences between juvenile plant reaction and adult plant reaction need to be recognised in tests of this kind.

BREAKDOWN OF RESISTANCE IN THE FIELD

Paka and T8 were amongst a range of genotypes introduced to the Cook Islands (South Pacific) from the Jamaica Plant Breeding Programme in 1979-80 for screening against *M. fijiensis*. Initially both were highly resistant to Black Sigatoka (Gonsalves 1987) with Paka being rated "immune" in some assessments (unpublished data). In 1989 both Paka and T8 became diseased. The rapid change in field reaction, and the reactions of plants in the glasshouse to strains collected before and after that event confirmed that there had been a change in the pathogen population to virulence on Paka (Fullerton & Olsen 1995). This was the first documented case of "breakdown" of resistance to *M. fijiensis*. Paka virulence is probably endemic to pathogen populations in the Papua New Guinea/ South Pacific region.

Yangambi km5, a triploid AAA of East African origin, is normally highly resistant to *M. fijiensis* under both field and laboratory conditions (Salle et al. 1990). When evaluated in Cameroon in 1988-89 it was highly resistant. By 1997 the variety was being severely diseased at all altitudes up to1250m (Mouliom-Pefoura 1999). This was the first documented case of breakdown of resistance in Yangambi km5.

The study by Fullerton & Olsen (1995) suggests that the same gene may control the resistance of Paka and Yangambi km5. Strains avirulent on Paka were also avirulent on Yangambi km5. When

Table 1. Reactions of selected strains of *M. fijiensis* on a range of *Musa* genotypes 1991 (after Fullerton & Olsen, 1995).

Strain/Country of origin	GN	T8	Paka	Yang	M. balb	Calc	Tuu	Pah	P. Berl	SF 215	P. Mas	AVP28 (P. Mas)
294 Samoa	4	1	2	2	4	5	3	5	2	4	2	4
674 Cook Islands	4	4	-	-	2	1	3	1	2	4	2	2
689 Cook Islands	4	4	5	4	4	1	1	5	3	4	2	4
638 Cook Islands	3	4	-	-	-	1	1	1	3	2	3	3
716 Cook Islands	4	1	-	-	-	-	1	2	2	3	1	5
743/1Cook Islands	-	-	-	-	5	5	-	4	-	5	-	-
743 Tonga	5	5	4	4		5	2	4	3	5	5	
298 PNG	5	4	-	-		4	1	4	5	5	5	4
299 PNG	4	2	-	-	1	4	1	-	-	4	4	-
300 PNG	5	1	-	-	-	1	1	1	4	4	4	-
326 PNG	5	5	-	-	-	5	3	4	5	5	5	-
330 PNG	5	1	-	-	-	5	1	3	5	5	4	4
297 PNG	4	3	-	-	-	4	1	-	-	5	4	-
310 PNG	5	2	-	-	-	5	1	4	4	5	4	4
302 PNG	5	1	-	-	-	5	1	4	4	5	5	5
318 PNG	5	2	2	2	-	5	1	3	4	5	5	-
321 PNG	4	4	-	-	1	1	1		3	4	3	-
475 PNG	5	2	-	-	-	1	1	5	5	5	5	-
722 Nigeria	4	1	-	-	-	1	1	4	3	5	4	-
730 Nigeria	4	4	-	-	-	0	1	-	2	5	4	-
548 Costa Rica	5	1	-	-	-	1	2	4	3	5	4	-
634 Honduras	4	5	-	-	-	2	3	1	4	5	4	-
719 Philippines	3	3	-	-	-	2	2	1	4	5	4	-

Reaction types:
Host perspective: 1= highly resistant ("immune"), 2 = moderately resistant, 3 = resistant, 4 = moderately susceptible, 5 = highly susceptible.
Pathogen perspective: 1 = avirulent, 2 = low virulence, 3 = low virulence, 4 = high virulence, 5 = very high virulence.
Abbreviations: GN = Grande Naine (AAA); Yang =Yangambi km5 (AAA); M. balb = *Musa balbisiana* (BB); Calc = Calcutta 4 (*M. acuminata* ssp. *burmanicoides*) (AA); Tuu = Tuu Gia (AA); Pah = Pahang (*M. acuminata* ssp. *malaccensis*) (AA); P. Berl = Pisang Berlin (AA); P. Mas = Pisang Mas (AA). PNG = Papua New Guinea
Blanks in table indicate no inoculation carried out.

challenged by strains virulent on Paka, Yangambi km5 expressed a typical susceptible response. Infertility in Yangambi km5 prevents direct genetic analysis of its resistance. Exposure to a greater range of strains having virulence on Paka would help confirm a relationship between their genotypes.

Implications of pathogenic variability in the search for disease resistant germplasm

Because of the biological limitations on breeding strategies, strain-specific resistance is likely to remain an important component of conventional breeding programmes. The studies of Fullerton & Olsen (1995) have demonstrated considerable pathogenic variability in *M. fijiensis* and that the resistance expressed by some of the banana genotypes is strain-specific and vulnerable to breakdown. Furthermore, resistance expressed by a genotype in one geographic locality may not necessarily ensure resistance in another.

Key elements of a strategy to minimise the risk of breakdown of resistance in new cultivars should include:

1. Identify new sources of resistance

The need to prospect for different sources of resistance is already widely recognised by banana breeders and pathologists (Anon. 1998; Anon 2000). Interest should remain centred on South East Asia, a major centre for *Musa* diversity and the source of Calcutta 4 and other potentially useful resistant subspecies such as *M. acuminata* ssp. *saimea* and *M. acuminata* ssp. *malaccensis*. Prospecting should include genotypes with a high degree of general resistance.

2. Genetic analysis to determine the numbers of genes involved in resistance.

The limited evidence available suggests that in at least some genotypes, more than one gene is involved in resistance to the Sigatoka leaf diseases. Resistance to yellow Sigatoka (*Mycosphaerella musae* Leach ex Mulder) in *M. acuminata* ssp. *zebrina* is considered to be partially dominant with multiple genes being involved (Vakili 1968). Shepherd (1990), using the same pathogen, considered that resistance to in the wild forms of *M. acuminata* was dependent, in part, on homozygous recessive genes. Ortiz & Vuylsteke (1992, 1993) have determined that resistance in Calcutta 4 is controlled by one major recessive allele (bsr1) and two independent recessive alleles with additive effects (bsr2 and bsr3). Until genetic markers are identified for major resistance genes in banana, conventional genetic analysis remains an essential step to indicate the potential durability of new sources of resistance.

3. Screen candidate resistant germplasm to strains of known virulence under controlled conditions.

Experience has shown that it may take several years of exposure of relatively large numbers of a particular genotype to detect virulence present at a low frequency in the pathogen population. The availability of strains of the pathogen with known virulence on specific genotypes offers an opportunity for rapid screening of new sources of resistance. Such screening is relatively easy to carry out, it can discriminate between strain-specific resistance and general resistance, and it can also indicate whether resistance in different genotypes is conferred by the same or different resistance genes. This should be viewed as an important adjunct to the genetic analysis of resistant genotypes.

4. Utilization of general resistance in breeding programmes.

Genotypes that express strain-specific resistance should be avoided unless it can be shown that there are other genes 'behind' the gene controlling the hypersensitive response. Strain-specific resistance is, however, a valuable future resource for use in marker assisted breeding. By targeting relatively high levels of general resistance (rather than immunity) in the diploid improvement phase of the programme, the progeny of the final crosses with susceptible triploids should inherit adequate levels of resistance. A number of progeny from the breeding programme in Nigeria, in which Calcutta derived diploids were used as the male parent, have been described as having 'partial resistance' (Ortiz & Vuylsteke 1998a, b, Ortiz et al. 1998), suggesting a 'dilution' of the original Calcutta resistance during the breeding programme. These cultivars still offer an acceptable degree of resistance. The 'amount' of general resistance needed to obtain a successful crop is not known and is likely to be environmentally dependent. In subsistence and small-scale commercial production, plants with moderate levels of disease would probably produce adequately for the intended purposes. At that stage other factors such as weevil borer, nematodes and viruses could present greater constraints to production than leaf disease.

5. Identification and utilisation of resistance to *Mycosphaerella eumusae* in breeding programmes.

The identification of a new pathogen, *Mycosphaerella eumusae* Carlier et al. (anamorph *Septoria eumusae* Carlier et al.) (Carlier et al. 2000) causing leaf disease of bananas in Southern Asia is a cause for concern. The symptoms are superficially similar to those of black leaf streak, and the disease can cause severe defoliation. The species is widespread in southern Asia (Southern India, Sri Lanka, Thailand, Malaysia, Viet Nam) and

has also been found in Mauritius and Nigeria. This disease must be regarded as a potentially serious threat to bananas worldwide. Two previous global epidemics of banana leaf diseases (*M. musicola* from 1913 and *M. fijiensis* from 1964) originated in the Asia/South Pacific area after the organisms had apparently remained quiescent in their areas of origin for generations. The possibility of a similar epidemic of *M. eumusae* cannot be discounted. Current collections and key breeding lines should be screened for resistance to *M. eumusae*, and resistance should be a regarded as a necessary attribute for new germplasm introduced to the breeding programmes. As with the Sigatoka diseases, the South East Asia region offers the best prospects for finding resistance to the *M. eumusae*.

Control of the Sigatoka leaf disease complex (*M. musicola*, *M. fijiensis, M. eumusae*) presents an ongoing challenge to subsistence farmers, commercial growers and breeders alike. The limited genetic diversity of *M. fijiensis* in areas of recent introduction (Carlier et al. 1996), its range of virulence in the Papua New Guinea/Pacific region (Fullerton & Olsen 1995), and the recent identification of *M. eumusae* in Asia and elsewhere (Carlier et al. 2000) indicate that banana growing countries will remain at risk not only from incursions of new species, but also from new strains of species already present. Broad-based, multigenic resistance should be the goal for all future *Musa* germplasm prospecting and utilisation programmes.

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