Monitoring in banana pest management*

M. J. Jeger†, J. M. Waller†, A. Johanson§ and S. R. Gowen**
†Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands, §International Mycological Institute, Bakham Lane, Egham, Surrey TW20 9TY, UK, and *Natural Resources Institute, Central Avenue, Chatham Maritime, Chatham, Kent ME4 4TR, UK, and *Department of Agriculture, University of Reading, Early Gate, P.O. Box 236, Reading RG6 6AT, Berkshire, UK

Monitoring in banana pest management is an important activity for commercial and/or smallholder banana production. It is required to detect the occurrence of newly introduced pest species, those which have previously been of minor importance, and for new variants which pose particular threats. Monitoring is important strategically in providing early warning of problems that may arise, and in some cases as a basis for pest management decisions within a cropping season. Information from monitoring can also serve public policy purposes, such as the need for eradication programmes and quarantine.

These different facets of monitoring are illustrated with reference to selected banana pests: the fungal pathogens causing leaf spots (Mycosphaerella spp.) and Panama disease (Fusarium oxysporum f. sp. cubense); parasitic nematodes (especially Radopholus similis) and the banana weevil (Cosmopolites sordidus). Copyright © 1996 Elsevier Science Ltd.

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Commercial banana production is vitally important to many developing countries and small islands in the tropics, and also smallholder cultivation of Musa spp. as a staple food for consumption in many parts of Latin America, sub-Saharan Africa and S.E. Asia. Indeed, commercial banana production represents only a small proportion of total global banana production (~10%) and the practices and needs of smallholder/subsistence farmers should therefore be kept in mind when considering the importance and consequences of banana pests and diseases. Unfortunately, although many studies or surveys have been made on pest and disease prevalence in the field, it has often been from the perspective of information for breeding new and improved commercial varieties, rather than for the improvement of local production and consumption. Thus much of the research efforts in the last 40 years or so is biased towards the commercial sector and may not be representative of the problems facing global Musa production.

The history of the banana industry has been closely linked to the pests and diseases of the crop. The replacement of Gros Michel by clones of a single variety, Cavendish, due to breakdown of resistance to race 1 of Fusarium oxysporum f. sp. cubense causing Panama disease has led to a situation where an extremely narrow genetic base, derived from selection rather than breeding, is now threatened by new pathogenic variants of this fungus and other species, placing the entire banana industry at some risk. On a more local scale, similar situations have arisen with Bluggoe plantain and Moko disease in the Caribbean and Central America. The historical background to these developments is provided in several key books and publications, notably those of Wardlaw (1972), Stover (1972a), Simmonds (1966), Stover and Simmonds (1987), Fullerton and Stover (1990), Plow (1990), Jeger et al. (1995) and Gowen (1995). The reality of the situation is that monitoring, especially in relation to new pathogen and pest variants becomes strategically important in providing an early warning of problems that will undoubtedly arise. Whether the commercial industry and/or small holder farmers will be equipped to respond to such challenges is at present an open question.

Monitoring for banana pests

The major pest constraints to banana production are: the fungal diseases, black Sigatoka (Mycosphaerella fijiensis), Panama disease (Fusarium oxysporum f. sp. cubense), and post-harvest rots usually involving Colletotrichum musae; the bacterial disease Moko (Pseudomonas solanacearum); the virus disease bunchy top (vectored by the banana aphid Pendalona nigrornerosa); and the invertebrate pests, burrowing nematode (Radopholus similis) and weevil borer (Cosmopolites sordidus).

For each of these pests different strategies have dominated pest management practices, including highly intensive chemical applications for black Sigatoka, national eradication programmes for Moko and bunchy top, screening germplasm for virus diseases, and international breeding and germplasm improvement programmes. Accounts of current practices for banana diseases and pests are given in Jeger et al. (1995) and

†Author to whom correspondence should be addressed
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Gowen (1995), respectively. Monitoring and early warning systems for some aspect of pest status have been practised for most of the above pests and will be discussed selectively in this paper. Molecular probes have been developed and used to distinguish Mycosphaerella spp. and to monitor the spread of black Sigatoka in E. Africa and the Caribbean, and to improve virus detection during quarantine. Biochemical assays have been developed as diagnostic tools to detect strains of *F. oxysporum* f. sp. *cubense* in east Africa, where Panama disease symptoms are prevalent in Uganda and the Kenya highlands, and Race 4 of the pathogen (to which commercial Cavendish cultivars are susceptible) in the sub-tropics. Fungicide insensitivity in *Mycosphaerella* and post-harvest pathogens is routinely assessed in the Windward Islands. The monitoring of fruit quality on arrival in importing countries provides rapid information on post-harvest diseases and possible problems in plantation pest management. Monitoring and legal controls for Moko disease to other Caribbean islands. Inspection and roguing of diseased plants in New South Wales has contained but not eradicated bunchy top. Contamination of newly planted areas with soil invertebrate pests, especially nematodes, poses a considerable threat given limitations on future use of nematicides such as non-volatile organophosphates/carbamates, and will require careful monitoring. Greater use should be made of micropropagated planting material certified free of invertebrate pests and pathogens, although this can lead to new problems if susceptibility to virus infection is enhanced.

To illustrate the achievements of monitoring in banana pest management, examples are given for the two major fungal diseases, black Sigatoka and Panama disease, and for nematodes and the banana weevil *Cosmopolites*. Much, of course, can be done to make better use of monitoring. There is a particular danger of pathogens which are currently of minor importance becoming significant on new cultivars (Waller et al., 1991). That these pathogens are 'minor' is just as much a function of host resistance as it is of pathogen aggressiveness. With new banana germplasm this feature needs close monitoring. There are many examples in the literature of the emergence of apparently new diseases on new or exotic crop cultivars shown to be caused by known pathogens previously of little importance. The resistance of wild germplasm to *Mycosphaerella* is not normally attacked by *M. musicola*. Although it has been reported, anecdotally, that black Sigatoka has replaced yellow Sigatoka within two years, wherever it has been introduced, this has not been proven, and is now doubted (Jones, 1990). The distribution of black Sigatoka in S.E. Asia and the Pacific is uncertain. Jones (1990) recently visited several countries in S.E. Asia to collect diseased material, and observed symptoms of black Sigatoka. Most of the major plantain and banana cultivars grown today are highly susceptible.

Good disease control is essential for export, as fruit from affected plants tends to ripen prematurely (Ramsey et al., 1990). Where the disease is not controlled, the leaves senesce faster decreasing the functional leaf area and thus decreasing yields. In addition, yield is reduced in areas with both types of Sigatoka leaf spot, as it can become necessary to harvest fruit at a lower grade (i.e. younger) in order to lessen the chances of premature ripening in transit (Stover, 1974; Stover and Simmonds, 1987).

The only practical means of control of black Sigatoka is by regular applications of fungicides, which are expensive and environmentally damaging. The cost of black Sigatoka control has been estimated as approximately three to six times greater than for yellow Sigatoka. For example in Honduras in 1984, black Sigatoka control was approximately 26% of the cost of producing a box of bananas. The cost of control in Central America, Colombia and Mexico from detection until 1985 was estimated to be over US$350 million (Stover and Simmonds, 1987).

**Monitoring Sigatoka leaf spots.** One of the major problems in the study and monitoring of the Sigatoka leaf spots is the difficulty of accurately identifying the pathogens. Although typical symptoms of the diseases are distinctive, if yellow Sigatoka is severe at certain stages of disease development it can be almost impossible to distinguish from black Sigatoka. Symptoms also vary depending on the cultivar and whether the plants have received oil and/or fungicide treatment. Unambiguous diagnosis of both diseases is also complicated by the presence of other pathogens found on banana leaves. In addition to other species of fungi, such as *Cladosporium*, causing leaf spots, two other species of *Mycosphaerella*, *M. musae* and *M. minima* are often present, and occasionally cause minor leaf spotting. In areas where *M. fijiensis* and *M. musicola* co-exist, it is difficult to distinguish the presence of both on a leaf by symptoms alone.

Confirmation of diagnosis by isolation of the pathogens can prove difficult. The major feature used to distinguish *M. fijiensis* and *M. musicola* is the presence or absence of a scar at the base of the conidium (Pons, 1990). Conidia are, however, not always visible on infected leaves, and in culture the fungi are slow-growing and sporulation may be both difficult to induce and readily lost after storage and repeated transfer. The sexual stages (perithecia) are morphologically identical, and similar to that of the other two...
Mycosphaerella species except in spor size. Also mature perithecia are not always present on necrotic leaf tissue and have rarely been produced in culture.

A technique for the rapid and accurate identification of these fungi would be of great value in monitoring for the black Sigatoka pathogen in areas which have so far been free of the pathogen, and in determining the distribution and spread of the pathogen in areas in which it has recently been introduced.

**Novel diagnostic techniques.** Novel approaches in molecular diagnostics have been applied at the Natural Resources Institute (NRI) to develop a technique for rapid and accurate identification of these fungi. This technique is based on the amplification of fungal DNA by the polymerase chain reaction (PCR). The technique can be used to differentiate between the species using DNA extracted from the fungi, and also, more importantly, using DNA extracted from infected banana leaf material. It enables a rapid, powerful and accurate detection technique and identification within a few hours of a leaf sample being received.

The four Mycosphaerella species found on banana have been differentiated by random amplification of polymorphic DNA (RAPD analysis) (Johanson et al., 1994; Johanson and Serccinivasaprasad, 1994). Although many random primers were found to be useful, one RC07 yielded a product specific for M. fijiensis and can be used as a species-specific probe. This product is also visible when infected banana leaf DNA is amplified using primer RC07, and has diagnostic potential (Johanson et al., 1994). In general, however, this technique is only of use when analysing DNA extracted from pure cultures of the fungus, and not when total DNA extracted from infected leaves is amplified because the primers also find homology in the banana genome.

A more reliable technique has also been developed, based on amplification of the ribosomal DNA of the fungi. Species-specific PCR primers were designed from the variable ITS1 region of the ribosomal DNA of M. fijiensis and M. muscicola. When these are used with standard primers in conserved regions of the rDNA, they produce a specific amplification product when fungal DNA is amplified by PCR. Using this method, fungal DNA can also be detected in infected material (Johanson and Jeger, 1993a,b; Johanson, 1994). This technique can be applied to a simple extract of infected banana leaf, and results are obtained within hours.

**Use of PCR diagnostics in monitoring for black Sigatoka.** Laboratory facilities have been set up at Kawanda Agricultural Research Station in Uganda and local staff trained in the principles and use of the PCR technique. A detailed laboratory instruction manual has been produced with an easy to follow protocol. These facilities have been used to survey local varieties of banana growing areas in Uganda for Mycosphaerella infection. Samples were collected from three farms in each of 23 areas, giving a representative sample of different regions where leaf spots have been recorded. DNA was extracted, and diagnostic PCR tests were made on approximately 40 samples. Where results were ambiguous, PCR was repeated. Although a detailed analysis of the results has not yet been completed (Tushemereirwe, unpublished data), it was clear that the PCR test was effective in differentiating between the Mycosphaerella species on the leaf samples. In addition, several samples which had previously been identified from symptoms as M. fijiensis, tested negative by PCR, and have since been confirmed as symptoms of virus infection. The PCR diagnostic test has performed extremely well under the laboratory conditions at Kawanda. The usefulness of the test was determined by the ability of PCR to identify the pathogens in samples that were impossible to diagnose by visual symptoms alone.

A laboratory is also being established at the Research Division of the Windward Islands Banana Growers Association (WINBAN) in St. Lucia. Black Sigatoka has not yet reached the Windward Islands, and constant monitoring is essential to investigate attempts to isolate and eradicate the disease should it appear. The recent occurrence of black Sigatoka in Jamaica (INIBAP, 1995) was confirmed by identification of M. fijiensis using the PCR test on material sent to NRI by the Jamaican Banana Board.

**Possibilities of eradication.** Several banana producing areas are still free of black Sigatoka, most notably the Windward islands. The question arises as to the possibility of eradicating the disease, should it become established, by a combination of roguing diseased plants and stringent quarantine measures. Virtually the only case study on this topic is provided by Jones (1990). Commercial banana production in Australia is free of black Sigatoka despite the Australasian–South East Asia region being both the centre of origin of the cultivated banana and probably also that of the Sigatoka diseases. Black Sigatoka is present in Papua New Guinea and has been found on islands in the Torres Straits which separate it from the Cape York Peninsula in North Queensland. In 1981, black Sigatoka was found in Bamaga at the north of the Cape York Peninsula and, because of the perceived threat to the commercial growing areas further south, an eradication programme was started for Bamaga and several of the islands in the Torres Straits. The region was proclaimed a quarantine area with no movement of banana material allowed to other parts of Australia. All bananas were treated with glyphosate, and no replanting was permitted until at least 6 months after the destruction of the crop. Healthy bananas were subsequently reintroduced. Despite apparent success on some of the Torres Straits islands, symptoms of black Sigatoka reappeared at Bamaga (Jones, 1984). Whether this reappearance was due to incomplete eradication in the first instance, or to other reservoirs of inoculum such as wild Musa banksii, or long-range wind dispersal of ascospores has not been established. Initially it was decided not to mount a second eradication programme, but to contain the disease through the gradual replacement of susceptible by resistant cultivars. This decision was reversed under pressure from the Queensland banana industry and a second eradication campaign began in 1989 but on this occasion only resistant cultivars were to be reintroduced.

It is also of considerable interest that as part of a wider attempt to ascertain risk from black Sigatoka, many other countries in the region were visited.
including Indonesia, Malaysia and the Philippines. Black Sigatoka was only found in the Philippines which is contrary to published information suggesting its widespread occurrence throughout south east Asia and the supposed displacement of *M. musicola* by *M. fijiensis* (Jones, 1990).

**Fusarium wilt**

*Fusarium* wilt of bananas caused by *F. oxysporum* f. sp. *cubense* (FOC), was first recorded from Australia in 1874 (Pegg and Langdon, 1987), but became notorious when it appeared in the Central American region around the turn of the century when it was given the name Panama disease. Ashby (1913) gave the first good description of the disease and the pathogen, but the first extensive studies were made by Brandes (1919). The disease was also recorded in Hawaii, South America, Asia and West Africa during the early 1900s. Although the disease seems to have been present in these areas for a long time, only causing concern when large commercial areas of the susceptible Gros Michel banana were planted, it was most likely carried with planting material from its centre of origin as a pathogen co-evolved with *Musa* in Asia. There is no evidence that the pathogen is present in virgin soils and some major banana producing areas have remained free of the disease for many years. Rather curiously, the disease has still not been recorded from most of the Pacific islands.

The devastation caused to the banana industry in the Central America region by Panama disease during the first half of this century prompted considerable research which has been comprehensively reviewed by Stover (1962). In the large commercial export-oriented banana growing areas the disease has been successfully controlled by planting resistant Cavendish varieties, but in areas where bananas and plantains are grown primarily for local consumption such as Africa, the disease is prevalent on exotic cultivars and causes substantial losses in some countries.

The symptoms of Panama disease are those typical of vascular wilts; affected plants initially show premature yellowing of the older leaves with progressive necrosis and collapse so that severely diseased plants have few erect leaves with most hanging down against the pseudostem. Eventually the unfurled heart leaf dies and the pseudostem collapses. The rate of disease progress is influenced by environmental factors such as soil conditions and temperature so that symptom expression may not always be complete on mature stems. Typically a purple/brown discoloration develops in the vascular strands in the rhizome and pseudostem from which the pathogen can be readily isolated. Other symptoms sometimes observed are chlorotic streaks and brown flecks of the inner surface of the petiole. Symptoms are seldom evident on suckers although these may be infected because the pathogen is systemic and spreads to all shoots on the same root mat. Spread between mats is slow and the main way in which the pathogen is distributed is by movement of infected planting material or contaminated soil and/or farm implements.

**Monitoring for FOC.** Reliance on symptoms for monitoring Panama disease present two basic problems. Suckers often do not show symptoms, yet these are the main source of planting material, and symptoms resembling the earlier stages of the disease can be caused by adverse cultural conditions and also by other pests and pathogens. Diagnosis has thus to start with the isolation of the fungus but to determine that the isolate is a special form of *F. oxysporum* which causes Panama disease presents another set of problems. There are no reliable morphological or simple cultural characteristics by which pathogenicity can be determined, and testing virulence is both time-consuming since symptoms develop slowly on inoculated plants and their expression is affected by various factors, although Sun and Su (1984) have shown that the disease can be reproduced by young plants under controlled conditions. Therefore more specialised mycological techniques are being developed to identify the pathogen.

**Pathogenic variability.** Pathogenic variability within FOC has led to its subdivision into races differentiated on their ability to cause disease on specific cultivars: races 1 and 2 are both widely distributed but are not virulent to Cavendish cultivars, whereas the rather anomalous race 3 is restricted to Central America and only attacks *Heliconia* spp. Race 4 is able to attack the Cavendish cultivars and some other cultivars susceptible to race 1. It is still restricted in distribution mainly to subtropical areas but is now thought to be present in S.E. Asia (Ploetz, 1995) and is causing concern. Meaningful monitoring of Panama disease requires that the particular races present are identified to ensure appropriate deployment of resistance.

An understanding of the variability within *F. oxysporum* and how this can be measured is crucial to the successful monitoring of Panama disease. Various techniques for assessing this variability have been reviewed by Ploetz (1990). *F. oxysporum*, has no known sexual phase so that the dynamics of variability are restricted, however, morphological variability occurs between and within isolates during growth on artificial media. There have been various attempts to relate particular cultural attributes of pathogenicity to bananas (Waite and Stover, 1960; Sun, Su and Ko, 1978), but with no consistency of success partly because disease development and expression is greatly affected by environmental conditions. FOC can also be divided according to vegetative compatibility groups (VCG). These are clonal populations separated by their inability to combine vegetatively and exchange genetic material asexually. FOC contains about 20 VCG (Ploetz, 1994); most of them occur in Asia, fewer in America or Africa which also indicates the earlier origin of the pathogen in Asia, but the most prevalent VCG are geographically and racially diverse. Ploetz (1994) has also indicated that electrophoretic karyotyping, RFLP and RAPD analyses group the VCGs into two main sections seemingly representing common lineages based on races 1 and 4. This suggests that the pathogen originally spread from one or two centers of origin in Asia rather than arising through selection from separate local populations of non-specialised *F. oxysporum*, although this does not explain the scattered distribution of race 4.

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Novel diagnostic techniques. The International Mycological Institute (IMI) is developing rapid diagnostic tests for identifying pathogenic strains in developing countries. Particular races within special forms of *F. oxysporum* can often be detected with particular techniques, for example pectinase isozyme patterns indicate that polygalacturonase patterns for race 4 or FOC appear to be unique, but no one test has so far been found which will identify all the variants within a special form (Rutherford, 1994). Thus a combination of tests including the use of VCG testing is required to cover the range of variability found within FOC. If a culture belongs to a recognised F-OC VCG group, this is an indication that it is pathogenic. However, the variability within the most common VCGs suggests that they are also likely to contain strains not pathogenic to banana. Mitochondrial DNA analyses can differentiate variants which correspond to pathogenic forms or races. For example, race 4 of FOC has unique mtDNA polymorphisms (Thomas et al., 1994); this and other variants can be detected using readily produced mtDNA probes (Bridge et al., 1995).

DNA based techniques which are able to identify critical lineage or biochemical groups might be the best laboratory indicator of pathogenicity. However, pathogenicity to *Musa* (or to other plant genera) does not seem to be strongly linked with many other characters and division of *F. oxysporum* into special pathogenic forms may not be the most natural way of subdividing the species.

**Future threats from FOC.** In practical terms, determining future threats from FOC requires more information on the variability of the pathogen, particularly the extent and distribution of race 4 of FOC. Currently this race is restricted to mainly subtropical areas where suboptimal conditions may help to predispose plants to the disease. Why this variant appeared in areas as widely separated as Taiwan, S. Africa, Queensland and the Canary Isles needs further elucidation before it is possible to predict future appearance elsewhere.

Race 4 does pose a threat to the banana industry and needs careful monitoring. In Africa, where non-export bananas and plantains are a major local food crop, Panama disease seems mainly confined to the exotic dessert cultivars but is prevalent in some East African countries (Ploetz, 1995). The unique local AAA African highland bananas in these areas are affected by a wilt disease that is not consistently associated with *F. oxysporum* infection (M. A. Rutherford and A. Kangire, pers. comm.). There is a need to monitor its occurrence closely and an etiological problem to be resolved.

**Invertebrate pests**

**Plant parasitic nematodes.** All bananas are susceptible to a number of species of plant parasitic nematodes. The most widespread and damaging species is *Radopholus similis* which has been transported throughout the tropics from its centre of origin in S.E. Asia. This nematode now infects most commercial banana plantations in Africa, Latin America and the Caribbean.

Although *R. similis* infects and can multiply on other crops and weeds (O’Bannon, 1977), it is possible to devise systems of break crops and fallows which will prevent the reproduction of this nematode, and therefore, it is theoretically possible to eliminate it from soil. However, the risk of recontamination from adjacent plantations is always present. It is most important that where new plantations (or introductions) of bananas are planned the source of planting material should be nematode-free, such as plants produced by tissue culture (Israeli et al., 1995). This is especially important for areas never previously cropped with bananas.

The International Mycological Institute (IMI) is developing rapid diagnostic tests for identifying pathogenic strains in bananas. For example, race 4 of *F. oxysporum* has unique mtDNA polymorphisms (Thomas et al., 1994); this and other variants can be detected using readily produced mtDNA probes (Bridge et al., 1995). DNA based techniques which are able to identify critical lineage or biochemical groups might be the best laboratory indicator of pathogenicity. However, pathogenicity to *Musa* (or to other plant genera) does not seem to be strongly linked with many other characters and division of *F. oxysporum* into special pathogenic forms may not be the most natural way of subdividing the species.

**Monitoring nematode populations.** Monitoring nematode populations in a perennial crop is complicated, particularly when damage thresholds are incompletely understood (Gowen and Queneherve, 1990). Assessment of root populations of nematodes or of lesion indices as described by Bridge and Gowen (1993) can form the basis for regular monitoring, and is done by commercial growers who practice chemical control (Gowen, 1979; Gowen and Queneherve, 1990).

In recent years the number of nematocides registered for use on bananas has declined as their toxicological hazards have been recognised. Also the repeated use of these compounds leads to the selection of a microflora that metabolizes the active ingredient and thus reduces persistence. The regular application of organophosphate and oxime carbamates in commercial banana plantations at rates of 2-3 g a.i. per plant (Gowen, 1979) is of increasing concern to environmental health authorities in many countries.

The nematode populations in roots may vary according to edaphic factors (Queneherve, 1988) and pathogenic variability of the nematode (Sarah et al., 1993). Of the group of banana cultivars grown for the desert export trade, all are susceptible; resistance or tolerance to *R. similis* has not yet been incorporated in export bananas, although the future prospects are promising (Gowen, 1993).
Banana weevil. The other major invertebrate pest of bananas is the curculionid, *Cosmopolites sordidus*. This weevil is only found on bananas and, like *R. similis*, originated in S.E. Asia and has been disseminated worldwide on planting material. There is debate concerning its importance as a banana pest, partly as a result of the difficulty in relating the numbers of adult weevils in the field and/or the extent of larval damage to the rhizomes, to yield loss (Mitchell, 1980). The need for and effectiveness of monitoring for weevils would be better appreciated if its status as a pest species was resolved in different banana cropping systems.

Monitoring weevil populations. Monitoring schemes based on trapping adults have been used as the basis for chemical control recommendations (Mitchell, 1978). However, in the field correct diagnosis can be difficult because of similarities in the symptoms with those caused by *R. similis*, *Pratylenchus* spp. and *H. multicinctus* (Gowen, 1995). Assessment of rhizome damage is a destructive procedure that can be done only on harvested plants, which imposes constraints on monitoring in some systems of banana cropping. Chemical treatments have been used widely and perhaps inadjudiciously, leading to insecticide resistance in some weevil populations (Mitchell, 1978; Collins et al., 1991), and where the pest is considered sufficiently important for insecticide application when monitoring of resistance is required.

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