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Review

Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease

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Water yam (*Dioscorea alata* L.) is the most widely cultivated yam species globally. The major limitation to the profitable and sustainable production of *D. alata* is its susceptibility to anthracnose disease. The availability of resistant varieties could potentially form the cornerstone of an integrated management strategy for yam anthracnose; however, anthracnose resistance breeding is hampered by the dearth of knowledge on pathogen identity and diversity. Four forms of *Colletotrichum* are now known to be associated with foliar anthracnose of yam: the slow-growing grey (SGG), the fast-growing salmon (FGS), the fast-growing olive (FGO), and the fast-growing grey (FGG) forms. The close phylogenetic relationship of the first three forms to reference isolates of *Colletotrichum gloeosporioides,* and the fact that only strains of these forms have been observed to induce typical anthracnose symptoms on *D. alata*, recently confirmed that *C. gloeosporioides* is the causal agent of yam anthracnose disease. The FGG form possibly represents a distinct, endophytic, species as indicated by morphological, biological and molecular criteria. Previous research emphasized epidemiology and control but limited progress was made in understanding yam anthracnose disease based on this classical approach. Molecular approaches have started to unravel the systematics and ecology of *Colletotrichum* strains associated with yam anthracnose, as well the population biology of *C. gloeosporioides* on yam. Sexual recombination is a likely mechanism contributing to the high genetic diversity of *C. gloeosporioides* in yam-based cropping systems. Studies have been initiated to understand the mechanisms that generate genetic variation in *C. gloeosporioides*, and to gain some insight into the biochemistry of the interactions between the pathogen and yam. Our thesis in this article is that integrating traditional and

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Abbreviations: ITS, internal transcribed spacer; DGGE, denaturing gradient gel electrophoresis; VCG, vegetative compatibility grouping; MP-PCR, microsatellite-primed PCR.

molecular approaches to understanding *C. gloeosporioides* systematics, epidemiology and population genetics will lead to a much better understanding of yam anthracnose disease, and thus to the development of effective and sustainable control measures. Research successes and challenges are discussed, as well as their implications for future studies on pathogen evolutionary potential, anthracnose resistance breeding, and the deployment of resistance genes.

Key words: Anthracnose, *Colletotrichum gloeosporioides*, *Dioscorea* spp., molecular markers, molecular systematics, population biology, resistance breeding, yam.

INTRODUCTION

Yams (*Dioscorea* spp.) constitute an economically important staple food for millions of people in the tropics and subtropics. West Africa accounts for about 95% of world production and 93% of the total yam production area (FAO, 2002). The two most important cultivated edible yams are white Guinea yam (*D. rotundata* Poir.) and water yam (*D. alata* L.). *D. rotundata* is indigenous to West Africa and represents the most important species in terms of volume of production while *D. alata*, which was introduced to Africa from Asia in the 16th century, is the most widely cultivated species globally. In comparison with *D. rotundata*, *D. alata* has superior characteristics for sustainable production, including high yield potential (especially under low to average soil fertility), ease of propagation, early vigour for weed suppression, and storability of tubers. Its major limitation in the field is the susceptibility of most cultivars to anthracnose disease that exerts a devastating impact on productivity. The deployment of durable host plant resistance in *D. alata* against yam anthracnose disease will contribute significantly to a high level and stability of field performance. Anthracnose (*Colletotrichum gloeosporioides*) causes leaf necrosis and dieback of yam vines, leading to a reduction in the effective photosynthetic surface area of the crop with a concomitant reduction in ability of the yam tuber to store food reserves. Epidemics that commence prior to or during tuber formation can have a tremendous effect on tuber yield. No measures that ensure sustainable control of anthracnose are available at present and the disease continues to cause considerable concern among yam growers. Successful control of anthracnose would encourage greater widespread cultivation and significant increases in overall production to meet the high local and overseas demand for yam.

National Agricultural Research Systems (NARS) in the Caribbean, Nigeria, India and the South Pacific conducted most of the early research on anthracnose in the 1960s and 1970s. More recently, the International Institute of Tropical Agriculture (IITA, Ibadan-Nigeria) has been at the forefront of yam anthracnose research in collaboration with NARS and advanced research laboratories. Following severe anthracnose epidemics in Nigeria in 1993, and the demise of the popular susceptible *D. alata* cultivars White Lisbon and Pacala in the Caribbean (McDonald et al., 1998; Ano et al., 2002), concerted effort has been made to develop programmes for the systematic breeding of yams for anthracnose resistance. Early research emphasized field evaluation of local and introduced yam germplasm for anthracnose resistance, epidemiology, and chemical control that has led to the development of fungicide resistance (Bayart and Pallas, 1994) besides being inappropriate for resource-poor farmers. Programmes for the systematic breeding of yam for anthracnose resistance were largely non-existent but have now been initiated in Africa at IITA, and in the Caribbean at the Institut National de la Recherche Agronomique (INRA – Guadeloupe). Knowledge of pathogen identity and population biology is crucial to the success of these breeding programmes.

This review will highlight the economic importance of yam anthracnose and provide a brief overview of the biology and ecology of anthracnose disease, followed by a summary of recent advances in the systematics and population biology of *C. gloeosporioides* associated with foliar anthracnose of yam. It concludes with perspectives on the future direction of yam anthracnose research. To our knowledge, this is the only review of advances in yam anthracnose research since that of A. O. Nwankiti and L. S. O. Ene, presented exactly 20 years ago at the sixth symposium of the International Society for Tropical Root Crops (ISTRC), Lima, Peru, in 1983 (Nwankiti and Ene, 1984).

IMPORTANCE OF YAM ANTHRACNOSE DISEASE

Anthracnose disease has had a dramatic impact on *D. alata* cultivation in regions such as the Caribbean where monocultures with a single popular susceptible variety are common. In this region, susceptibility to anthracnose has made it virtually impossible to grow the popular varieties Pacala and White Lisbon, and anthracnose is considered the single most important factor responsible for the decline of yam production (McDonald et al., 1998; Ano et al., 2002). Nwankiti and Ene (1984) reported that anthracnose might cause yield reduction of up to 80% in Nigeria. Green (1994) recorded losses in excess of 85% while Mignucci et al. (1988) reported losses exceeding 90%. Farmers in the Caribbean can no longer rely on the

hitherto effective benzimidazole fungicides due to the development of fungicide-resistant *C. gloeosporioides* strains (Bayart and Pallas, 1994). On-farm yield-loss studies in three agroecologies within the yam production zone of West Africa showed that the impact of anthracnose on yield is highly dependent on time of disease onset and the prevalent environmental conditions (Green, 1994; Green, 1998). *C. gloeosporioides* was recently found to be associated with deep-seated (systemic) infection of botanic seeds of yam (Abang, 1997). Yam breeders do report instances where it has proved impossible to obtain viable seeds from parental genotypes because of the high flower abortion and low fruit set associated with the disease. Although the exact role of infected seed in the epidemiology of yam anthracnose is yet to be ascertained, it poses a serious problem in the international distribution and exchange of yam (seed) germplasm since materials have to be certified disease-free. Furthermore, the disease is implicated in the erosion of genetic diversity in large-scale field collections of yam germplasm (Orkwor and Asiedu, 1995). *Colletotrichum gloeosporioides* is known to cause 'dead skin' disease in the Caribbean, in which severely affected tubers fail to sprout and become rapidly infected with other pests and pathogens (Green and Simons, 1994). The pathogen is believed to play a role in tuber biodeterioration in Nigeria; however, 'dead skin' symptoms have not been reported. Anthracnose occurs wherever yam is grown but extreme temperatures in parts of northern Nigeria appear unfavourable for disease development (Nwankiti and Ene, 1984; Green, 1998). In areas where anthracnose is not currently considered a constraint, there is a risk of higher yield losses in the future, as the trend towards shorter fallows increases and as new hybrids replace a wide spectrum of local landraces.

BIOLOGY AND ECOLOGY OF YAM ANTHRACNOSE

The pathogen causing yam anthracnose was first described as *Gloeosporium pestis* Massee from yam in Fiji (Massee, 1908 cited in Winch et al., 1984). It was later reported from *D. alata* in India (Prasad and Singh, 1960; Singh et al., 1966) and subsequently classified as *C. gloeosporioides*. Abang et al. (2001) described four forms of *Colletotrichum* associated with foliar anthracnose of yam in Nigeria: the slow-growing grey (SGG), the fast-growing salmon (FGS), the fast-growing grey (FGG), and the fast-growing olive (FGO) forms. Isolates of the four forms were identified as *C. gloeosporioides* based on morphology. Variation in growth rate, and conidial and appressorial morphology were important criteria for differentiating the *Colletotrichum* strains. Forty one percent of all isolates (100 % of the FGG isolates) produced abundant perithecia in culture, suggesting that these isolates may be able to reproduce via the teleomorph, *Glomerella cingulata*, in yam fields. The SGG isolates did not produce the teleomorph in culture; however, failure to observe the sexual state does not necessarily imply the inability to produce it but may simply reflect the absence of conditions optimal for the production of the teleomorph by certain strains. Thus, while traditional methods for species delineation within *Colletotrichum* and for discrimination of sub-populations within species rely primarily on morphological characteristics, the strong influence of the environment on these traits has made their use unsatisfactory. Isolates thought to belong to *C. gloeosporioides* were found to be highly variable in morphology and virulence (Abang, 1997; Abang et al., 2001), and some FGS isolates with fusiform conidia appeared conspecific with *C. acutatum*. Moreover, *C. gloeosporioides* strains were isolated from leaves, stems, tubers and botanic seeds of yam (Green and Simons, 1994; Abang, 2003), raising questions as to whether the same *Colletotrichum* species is responsible for disease symptoms on the different plant parts.

The presence of several pathogenic fungi on the yam phylloplane led Amusa et al. (1996) to suggest that yam anthracnose is a 'disease complex', requiring the concerted action of a number of fungal pathogens for significant symptom development. In virulence studies under controlled environment conditions, however, Abang (1997) showed that a single slow-growing grey (SGG) isolate of *C. gloeosporioides* is capable of causing 100% leaf abscission and premature death of up to 76% of inoculated plants. Earlier reports showed that the disease was severe only on *D. alata* but more recent investigations have revealed that the disease is prevalent and severe on most of the widely cultivated species of yam (Akem and Asiedu, 1994). Indeed, Nwankiti (1982) reported an outbreak of anthracnose on *D. rotundata*, a species usually considered resistant to *C. gloeosporioides*. This raises the question as to whether the symptoms observed on *D. rotundata* were typical anthracnose symptoms caused by *C. gloeosporioides*, or if other leaf spots caused by fungi such as *Curvularia* sp. (Amusa et al., 1996; Green, 1998; Akem, 1999) were more important in the epidemic on *D. rotundata*.

The terms 'scorch', 'Apollo', 'anthracnose/blotch', 'leaf and stem blight', and 'yam dieback' have been used to describe foliar diseases on yam. This confusion in nomenclature arises from the fact that a range of diverse symptom-types ('tarspot', 'splash' lesions, 'pin-prick' lesions, leaf blight, stem blackening and shoot dieback) is associated with foliar anthracnose on yam (Green, 1998). This may have led to *C. gloeosporioides* being considered responsible for epidemics on *D. rotundata* that were caused by other fungi. There was, therefore, the need to clarify the symptomatology and aetiology of foliar diseases on yam, as this has serious implications for the development of disease control measures. In a survey to determine the prevalence of yam foliar diseases

across farmers' fields in the yam belt of Nigeria, *Colletotrichum* sp. was identified as the major pathogen associated with yam dieback and foliar necrosis (Akem, 1999). Other microorganisms isolated from the samples were: *Botryodiplodia* spp., *Curvularia* spp., *Pestalotia* spp. and *Fusarium* spp. Following artificial inoculation of *D. alata* clones with these pathogens singly and/or in combinations, only *C. gloeosporioides* reproduced typical symptoms of yam dieback and necrosis when inoculated alone. The predominance of *Colletotrichum* sp. from sample isolations and the reproduction of typical symptoms with *C. gloeosporoides* were considered as proof that *C. gloeosporoides* is the main causal agent of yam dieback of *D. alata*. Akem (1999) argued that the disease on *D. alata* should be called "yam anthracnose", to avoid confusion in symptom nomenclature arising from the use of several common names.

Winch et al. (1984) provided a detailed description of the different symptoms produced by *C. gloeosporioides* and how these develop on *D. alata*. They reported that pinpoint lesions (<2 mm diameter) may represent the earliest stage of infection on young leaves or the final stage of invasion of more mature, fully expanded, leaves and that these appear more frequently on the adaxial than on the abaxial leaf surface. Typical anthracnose lesions are dark brown to almost black, irregular or roughly circular and 2-20 mm in diameter. Infection generally extends through the lamina but is sometimes restricted laterally by the veins. Lesions may coalesce or, on young leaves, expand to form large blotches, and 'shot-holes' may develop as necrotic tissue falls away in larger lesions. Early infection may result in premature death of the plant. Winch et al. (1984) observed an unusual superficial blackening of upper leaf surfaces of some *D. alata* cultivars. Melanin-like compounds were found to be associated with appressoria of *C. gloeosporioides*, but there was little evidence of fungal penetration into host cells (Winch et al., 1993).

Two distinct symptom-types were observed under controlled environmental conditions following inoculation of *D. alata* with different *C. gloeosporioides* strains (Abang, unpublished data). The first was characterised by discrete, circular and generally non-coalescing dark brown/black necrotic lesions, usually surrounded with a bright chlorotic halo, while the second could be distinguished by diverse, irregular and generally coalescing chlorotic patches, with only superficial browning of the leaves. These differences in symptom-type were independent of the pathogen strain present but appeared to be host genotype-dependent. The severity of both symptoms was strongly influenced by the cultivar x strain interaction.

The optimum temperature for growth and sporulation of the pathogen is known to vary between 26^0C and 32^0C (Wharton, 1994). Spore viability and germination are extremely sensitive to atmospheric humidity; at 99 % relative humidity (r.h.), germination was half that at 100% r.h. and was found to be negligible below 97% r.h.. Lesion formation is, therefore, dependent on periods of 100% r.h. or leaf wetness of sufficient duration to allow for spore germination and host penetration. Sweetmore et al. (1994), Green (1994) and Ekefan (1996) investigated the temporal and spatial aspects of anthracnose development on yam. Epidemics of anthracnose were found to be initiated from randomly distributed foci and secondary spread of the disease from these foci resulted in an aggregated spatial pattern that ultimately became uniform. The rate of progression through this sequence of spatial patterns was affected by the susceptibility of the cultivar, age of leaf, age of vine, stage of the epidemic, rainfall and agronomic practices such as fungicide application (Sweetmore et al., 1994; Wharton, 1994).

Isolates of *C. gloeosporioides* from a three-year field survey in Nigeria were examined using an *in vitro* assay to determine the incidence of resistance to benomyl, thiabendazole and prochloraz (Abang, 2003). All 217 *C. gloeosporioides* isolates were classified as sensitive to the three fungicides; however, there was a tendency towards resistance to thiabendazole and prochloraz. Twenty-seven isolates (12.4%) showed 11-15% growth on thiabendazole-amended medium relative to the control, while 2.3% of the isolates showed 16-20% growth on the fungicide-amended medium. On prochloraz-amended medium, 5.5% of the isolates showed 11-15% growth and one isolate showed up to 25% growth on this medium (just below the cut-off point for resistance). These results suggest that a low level of fungicide resistance exists in Nigeria but that strains with apparent resistance have low fitness and occur at a low frequency. The use of fungicides is likely to increase with intensification of yam cultivation in West Africa. Field experiments are needed to determine the choice of fungicides and to minimise the selection pressure on fungicide-resistant strains.

Kolattukudy et al. (2000) have reviewed advances in understanding the molecular events in the early infection processes involved in the interaction between *C. gloeosporioides* and its hosts. Most of these studies focused on anthracnose of fruits. Negligible information is available on the mechanisms by which the pathogen penetrates yam leaves. Penetration through natural openings such as stomata (Nwankiti and Okpala, 1984) and wounds is possible, however, wounding did not increase symptom severity on *D. alata* (Abang et al., 2001). The finding that wounds did not facilitate infection was in agreement with the observation that *C. gloeosporioides* gains entry into the host predominantly by direct penetration of the cuticular barrier (Nwankiti and Okpala, 1984; Kolattukudy et al., 2000). In spite of its ability to penetrate the cuticle directly, mature leaves with a well developed cuticle are known to be considerably more resistant to attack by *C. gloeosporioides* than are younger leaves (Sweetmore et al., 1994; Abang, 1997).

Similar to the use of early planting to avoid the onset of epidemics from coinciding with the stage when plants have predominantly young leaves, breeding for a thick cuticle in leaves of young *D. alata* plants may contribute to combating the disease.

Plumbley and Sweetmore (1994) have shown that resistance to anthracnose in *D. alata* may be controlled by phenolic antifungal compounds, suggesting that the production of phenolases (tyrosinase) is a likely mechanism by which resistance is overcome by some *C. gloeosporioides* strains. Abang (2003) surveyed over 200 *C. gloeosporioides* isolates and found that most isolates tested positive for putative tyrosinase activity; however, a correlation between tyrosinase activity and virulence was not found. In addition to enzymes that can degrade cell walls and macerate yam leaf tissues, phytotoxic secondary metabolites (pathotoxins) are also associated with the invasion of tissues by *C. gloeosporioides*. Amusa et al. (1993) and Ahoussou (1989, cited in Moura-Costa and Mantell, 1993) have extracted phytotoxic substances from *C. gloeosporioides*-infected yam leaves, which induced necrotic lesions similar to that produced by the pathogen on yam leaves. The toxin extracted by Amusa et al. (1993) gave a fluorescent band similar to that produced by toxic metabolites of the pathogen in culture. Alleyne (2001) found that *C. gloeosporioides* toxin induced electrolyte leakage in yam cell suspension cultures, a mechanism that may explain its toxicity. Ahoussou (1989, cited in Moura-Costa et al., 1993) and Alleyne (2001) attributed the phytotoxicity of toxin from yam isolates of *C. gloeosporioides* to glycoprotein-like compounds. The purification and characterization of a highly toxic fraction by Abang (2003) revealed the presence of a low molecular weight compound, suggesting that the compound purified was not a glycoprotein. Autoclaving did not destroy the toxin; hence, the toxin was considered to be thermostable (Abang, 2003). Although the structure of three compounds purified by Abang (2003) could not be identified, the isolation protocols described and the characteristic information about their UV and NMR spectra may facilitate the identification of these compounds in future studies. It has been suggested that *C. gloeosporioides* toxins could be used to screen for anthracnose-resistant varieties (Moura-Costa et al., 1993). The toxins may prove to be a beneficial tool in future studies on chemotaxonomy, host-pathogen interaction and on the nature of anthracnose resistance in yam.

MOLECULAR SYSTEMATICS OF YAM-ASSOCIATED *COLLETOTRICHUM*

The unambiguous identification of *Colletotrichum* species and the definition of sub-populations responsible for epidemics is vital for developing and implementing effective disease control strategies. For instance, *C. gloeosporioides* and *C. acutatum* differ in their sensitivity to fungicides such as benomyl. Implementing a spray program where a mixed population exists without accounting for their differential sensitivity may result in a shift in population ratio (Freeman et al., 1998). *C. gloeosporioides* is widely accepted as the causal agent of yam anthracnose based on morphological criteria (Singh et al., 1966; Winch et al., 1984) but such criteria have often proved inadequate for differentiating *Colletotrichum* species (Brown et al., 1996). The current inaccuracies in identifying *C. gloeosporioides* and defining sub-specific groupings within the pathogen solely by using morphological criteria have been largely overcome by the use of molecular methods for species and sub-specific differentiation within *Colletotrichum*. Multiple morphologically indistinguishable *Colletotrichum* species are commonly associated with a single host (Freeman et al., 1998). Due to this complicated situation, a *Colletotrichum* species-host combination involved in a given anthracnose incidence alone is often insufficient as a diagnostic indicator of disease aetiology. Although *C. gloeosporioides* can infect different yam plant parts (Green and Simons, 1994; Abang et al., 2001), the question as to whether more than one *Colletotrichum* species is responsible for different anthracnose symptoms was hitherto not addressed. The diversity of disease symptoms and forms of the pathogen found on yam (Green, 1998; Thottappilly et al., 1999; Abang et al., 2001) raised questions whether more than one *Colletotrichum* species is involved as causal agent of a disease complex. *Colletotrichum* isolates from yam produced conidia which became septate upon germination indicating that they were not forms of the *C. orbiculare* aggregate species (Bailey et al., 1996). Still, uncertainty remained regarding the possible presence of other species having straight/cylindrical conidia and 'atypical' strains of *C. acutatum* (Brown et al., 1996).

Molecular identification of *C. gloeosporioides*

In view of the high morphological similarities between *C. gloeosporioides* and several other *Colletotrichum* species (Brown et al., 1996), a biochemical and molecular approach was used to resolve the taxonomic status of the yam anthracnose pathogen (Abang, 2003). The clarification of the systematics of *Colletotrichum* strains infecting yam is a necessary precondition for studies on the population structure of the yam anthracnose pathogen. The reaction of monoconidial cultures on casein hydrolysis medium (CHM), fungicide sensitivity, 18S rDNA polymorphism, PCR-RFLP and sequence analysis of the internal transcribed spacer region of the ribosomal DNA (ITS 1-5.8S-ITS 2) were used to ascertain the identity of the yam anthracnose pathogen(s). All *Colletotrichum* isolates from yam could be distinguished

from *C. acutatum* by the absence of protease activity on CHM. *Colletotrichum acutatum* reference isolates were clearly distinguished from *C. gloeosporioides* isolates obtained from yam based on their resistance to benomyl (>35% growth on benomyl-amended medium relative to the control), indicating that none of the yam isolates belonged to *C. acutatum*. Application of denaturing gradient gel electrophoresis (DGGE) to PCR-amplified 1.65 Kb 18S rDNA fragments clearly differentiated yam *Colletotrichum* isolates from *C. acutatum* (Fagbola et al., 2001), confirming that *C. acutatum* is not implicated in yam anthracnose disease in spite of the presence of FGS isolates morphologically similar to *C. acutatum* (Abang et al., 2001). FGG isolates produced unique ITS RFLP banding patterns, while FGS, FGO and SGG isolates produced RFLP patterns identical to those of *C. gloeosporioides* reference isolates but distinct from other *Colletotrichum* species. Restriction fragments generated by the endonuclease *Alu*I, *Hha*I and *Hae*III were useful for rapid differentiation of FGG isolates from other forms of *Colletotrichum* from yam (Abang et al., 2002). Sequence analysis of the ITS1 and of the entire ITS region further revealed high similarity among the SGG, FGS, and FGO isolates (98-99% nucleotide identity), with 97% to 100% identity to reference isolates of *C. gloeosporioides*. Less than 93% similarity of these isolates to *C. lindemuthianum* and *C. acutatum* was observed (Abang et al., 2002).

On the basis of the 'species' definition by Bailey et al. (1996), it was concluded that the SGG, FGS and FGO strains are all forms of *C. gloeosporioides*. Morphologically, these isolates fit the description of *C. gloeosporioides* (Mordue, 1971), which was subsequently confirmed by their response to benomyl, reaction on casein hydrolysis medium (CHM), and by ITS sequence analysis. Only these strains of *Colletotrichum* induced typical anthracnose symptoms in *Dioscorea*, hence it can be stated that *C. gloeosporioides* is the causal agent of anthracnose disease of yam. *C. gloeosporioides* isolates infecting yam in Nigeria were found to be closely related to isolates causing anthracnose in other hosts and geographical locations. FGS, SGG and FGO strains had high ITS sequence similarity to *C. gloeosporioides* infecting yam in Barbados (Sreenivasaprasad et al., 1996). FGO isolates were found to be identical to the FGS form based on both the ITS and 18S rDNA analyses, suggesting that the FGO isolates may be variants of the morphologically heterogeneous FGS group.

Teleomorphic and anamorphic isolates of the FGS, FGO and SGG strains clustered within a single rDNA sequence-defined group, providing evidence for an integration of mitosporic and meiosporic isolates in the species concepts of *C. gloeosporioides*/*G. cingulata* (Abang et al., 2002). This emphasizes the complex evolution of the ascomycetes, and indicates that the separate naming of the teleomorph and anamorph is probably obsolete. In spite of its robustness, the ITS sequence so far used to infer phylogenetic relationships among yam *Colletotrichum* strains represents only a small proportion of the total genome, and there is the risk of recreating gene trees rather than species trees. Sequence data from other phylogenetically informative regions of the genome need to be analysed to provide independent evidence of lineages and to critically evaluate species-level systematics within *Colletotrichum*.

The FGG form: A new *Colletotrichum* species from Nigeria?

The FGG group of isolates, morphologically described as *C. gloeosporioides*, formed a distinct ITS RFLP group and showed only limited (<86 %) ITS1 sequence similarity to *C. gloeosporoides* reference isolates. Isolates of the FGG form did not cluster with any previously described *Colletotrichum* species. In fact, FGG isolates appeared more distantly related to *C. gloeosporioides* than *C. acutatum* and *C. lindemuthianum*. Although this FGG group produces an anamorph similar to *C. gloeosporioides*, differences in colony characteristics, conidial, ascospore and appressorial morphology as well as virulence, separated it from other forms of *Colletotrichum* from yam. Ogle et al. (1986) described a teleomorph of *Colletotrichum* from *Stylosanthes* with characteristics similar to the FGG isolates and considered it to be a taxon distinct from *C. gloeosporioides/G. cingulata*. The taxonomic uncertainties with *C. gloeosporioides* extend also to its teleomorph *Glomerella cingulata*, and it is doubtful if the two names refer to the same fungus.

In tests of vegetative compatibility, Abang et al. (in press) found that FGG isolates were incompatible with SGG or FGS isolates. Furthermore, high performance liquid chromatography (HPLC) analysis of partially purified toxins produced similar chromatograms for the SGG and FGS isolates. In contrast, two toxin fractions corresponding to major peaks were conspicuously absent in the chromatogram of FGG isolates (Abang, 2003). FGG isolates are typically avirulent/weakly virulent in contrast to the FGS and SGG isolates that produce mostly moderate to severe anthracnose symptoms on yam (Abang et al., 2001). The FGG form has so far been isolated from *D. alata*, *D. rotundata* and *Calapogonium mucunoides*, indicating that it occurs on both yam and non-yam hosts (Abang, 2003). The association of this group of isolates with yam may be simply endophytic. Nevertheless, fungi commonly found as symptomless colonists may also cause disease (Bills, 1996). Taken together, differences in morphology, virulence, vegetative compatibility, and their distant phylogenetic relationship to other *Colletrichum* species, indicate that the FGG form is distinct from *C. gloeosporioides* and may represent a new *Colletotrichum* species. Analysis of sequence data

from multiple loci and further pathogenicity testing of larger samples of FGG isolates will help clarify the present taxonomic uncertainties surrounding this form of *C. gloeosporioides* on yam.

POPULATION BIOLOGY OF *C. gloeosporioides* FROM YAM

The integration of epidemiology and population genetics into a more unified approach – population biology – has been advocated as distinctly advantageous to solving practical plant pathology problems (Milgroom and Peever, 2003). In the following section we show how concepts of population biology can be applied at the strategic and tactical levels for anthracnose disease management.

Tracking pathogen populations

Yam is vegetatively propagated and planting material (yam tuber), which is an important source of *C. gloeosporioides* inoculum, is frequently exchanged within and across national borders in West and Central Africa. Only the SGG form has been observed to cause defoliation and premature death of inoculated plants (Abang, 1997; Mignouna et al., 2001). The threat of the spread of the aggressive SGG strain in this region must be urgently addressed not only on account of the virulence of this strain, which appears to be linked to its ability to produce highly toxic metabolites (Abang, 2003), but because of its epidemiological significance. The SGG strain was initially thought to be restricted to the humid forest zone of Nigeria but was recently isolated from severely attacked yam in the southern guinea savanna (Abang, 2003). Molecular differentiation of SGG and FGS populations using genetic markers will facilitate epidemiological studies (e.g. genotype tracking), as well as assist breeders develop improved strategies for resistance breeding against both pathogen populations (Mignouna et al., 2001; Mignouna et al., 2002a, b).

PCR-RFLP of the entire ribosomal DNA ITS1-5.8S-ITS2 region did not reveal any polymorphism between SGG and FGS isolates (Abang et al., 2002). Thottappilly et al. (1999) attempted molecular differentiation of SGG and FGS isolates based on RAPD analysis but failed to clearly distinguish the two morphotypes. FGS and SGG isolates were recently found to be vegetatively incompatible, indicating that the groups constitute distinct genetic sub-populations within *C. gloeosporioides* infecting yam (Abang et al., in press). An alternative approach was to target the small subunit (SSU, 18S) rDNA gene and analyse 18S sequence variation among the fungal isolates using DGGE. Application of the DGGE technique to 1.65 Kb SSU rDNA fragments was shown to be an efficient method for differentiating the SGG and FGS forms of *C. gloeosporioides* associated with anthracnose disease of yam in Nigeria (Fagbola et al., 2001). Because electrophoretic behaviour directly reflects differences in nucleotide composition of the fragments screened, the assay provided clear evidence of polymorphism among the taxonomic entities investigated. Genetic analysis based on 52 microsatellite-primed PCR (MP-PCR) markers revealed highly significant differentiation between the SGG and FGS populations on yam (G_{ST} = 0.22; Nei's genetic identity = 0.85; θ = 0.28, $P<0.001$), again indicating that the SGG and FGS morphotypes represent genetically differentiated populations (Abang, 2003). Taken together, distinct morphological and virulence phenotypes, complete vegetative incompatibility in complementation tests, high genetic differentiation and 18S rDNA polymorphism are consistent with SGG and FGS strains representing two separate evolving populations of *C. gloeosporioides*. Similar to the case of the FGG isolates, further genetic characterization would clarify the exact taxonomic status of the SGG isolates. Until that is done, we prefer to consider FGS and SGG isolates as representing two genetically distinct populations of *C. gloeosporioides* infecting yam.

The SGG type has, to our knowledge, not been reported from any other yam-growing region of the world. To facilitate studies of the origin and global distribution of the two pathogen genotypes, molecular tools such as specific PCR primers should be developed based on the SSU rDNA sequences that discriminate the defoliating SGG isolates from the FGS isolates. This will also enable the monitoring of the spread of the SGG population in Nigeria where this aggressive form is now spreading (Abang, 2003). Specific PCR primers would be used to perform amplifications directly on DNA extracted from crude field samples without the need to obtain pure isolates. This will allow the study of a greater number of samples in order to obtain a better knowledge of the biology and the epidemiology of these *C. gloeosporioides* populations on yam.

Sources of inoculum

Identifying and targeting the source of primary inoculum is necessary because management strategies such as the use of foliar fungicides aimed at polycyclic infections, may not be feasible in certain situations. The most important sources of *C. gloeosporioides* inoculum are infested crop debris (Green, 1994), infected tubers (Adebanjo and Onesirosan, 1987; Simons and Green, 1994) and alternative hosts (Simons, 1993; Green, 1994). Abang (1997, 2003) obtained both FGS and SGG isolates from dissected botanic seeds that were surface-sterilized and plated on culture media but the incidence of infection in seed lots and the efficiency of *C. gloeosporioides* transmission through seed remains to be

established. *C. gloeosporioides* was previously thought to survive in soil but Ekefan et al. (2000) showed that survival in soil is unlikely. Microbial antagonism appeared to play a significant role in the apparent non-survival of *C. gloeosporioides* in soil. The importance of infected tubers as a source of inoculum was established in epidemiological studies, which found a correlation between infected tubers sown and the amount of foliar disease subsequently observed. The most direct evidence of tuber or seed transmission will come from studies combining molecular genetic and epidemiological analysis of disease progress, whereby "marked" *C. gloeosporioides* genotypes are tracked from inoculated seed/tuber to foliar epidemics and then to the next generation of infected seed/tuber in the field.

Recently, Abang (2003) used a population genetic approach and hypothesized that ascospores are a dominant source of inoculum for anthracnose epidemics on yam in Nigeria. These conclusions were based on observation of *Glomerella cingulata* fruiting bodies on severely infected senescent yam leaves in Nigeria, finding extremely high levels of genotypic diversity, lack of subdivision in *C. gloeosporioides* populations, and random spatial patterns of anthracnose disease (Ekefan, 1996), all of which are consistent with sexual reproduction, long-distance dispersal of ascospores, and ascospore inoculum contributing significantly to secondary cycles of the disease. Additional studies on the relative contribution of asexual propagation, sexual reproduction and immigration using the type of mark-release-recapture techniques advocated recently (McDonald and Linde, 2002a, b) are now warranted to further test hypotheses concerning the evolutionary potential of the yam anthracnose pathogen.

C. gloeosporioides host specificity, genetic structure and evolutionary potential

The study of the genetic structure of *C. gloeosporioides* populations on yam might help resolve such issues as whether isolates found on *D. alata* are genetically related to those infecting other *Dioscorea* and non-yam species. The genetic structure of a pathogen population is likely to affect the pathogen's ability to evolve in response to control measures, such as the deployment of resistant varieties or the application of fungicides. Infact, knowledge of the diversity of pathogen populations is a prerequisite for effective resistance breeding as it ensures that early-generation breeding lines are screened against a wide range of genotypes in the pathogen population.

Analysis of *C. gloeosporioides* from yam in Nigeria and the Caribbean using RAPD markers demonstrated that there is a much higher level of genetic variability than pathogenic variation among isolates (Thottappilly et al. 1999; Alleyne, 2001). Studies of vegetative compatibility grouping (VCG) also revealed high VCG diversity in *C. gloeosporioides* from yam, with multiple genotypes occurring in the same lesion (Abang et al., in press). The underlying mechanism that produces such high levels of variability in the yam anthracnose pathogen is unclear. The sexual state of *C. gloeosporioides* (*Glomerella cingulata*) does not have a recognized role in anthracnose epidemics on yam, although it has been observed on old senescent yam leaves in Guadeloupe and Nigeria (Toribio et al., 1980; Abang, 2003).

Recently, simple sequence repeats have been used in microsatellite-primed (MP) PCR (Abang, 2003) to assess genotypic diversity and genetic differentiation among populations of *C. gloeosporioides* originating from different yam species (*D. alata*, *D. rotundata*, *D. dumetorum*) and non-yam hosts (mango and citrus) in three agroecological zones of Nigeria. MP-PCR is considered more robust than conventional random amplified polymorphic DNA (RAPD) analysis, because longer primers are used for MP-PCR than for RAPDs. This improves reproducibility and avoids spurious amplification based on partial primer binding. Only isolates classified as slow growing grey (SGG) and fast growing salmon (FGS) were included in the study. We found high genetic diversity and a high level of gene flow between geographically distant populations (Abang, 2003).

The results of virulence, vegetative compatibility and MP-PCR analyses showed that the pathogen population consists of many intermixed genotypes, suggesting that they are the product of recombination of virulence, vegetative compatibility and other loci that occurs during sexual reproduction. Outcrossing that occurs in the unusual and complex mating system of *G. cingulata* (unbalanced heterothallism) is thought to give rise to offspring with novel genetic combinations. The high genetic diversity reported in this study could also be explained by assuming that virulence on yam may have been acquired by a large number of genetically distinct strains. West Africa is one of the centres of origin of yam (Coursey, 1973), and is thus a presumed centre of diversity of its pathogen *C. gloeosporioides*. This can influence pathogen variability because diversity of *C. gloeosporioides* has been shown to be extensive at sites where native or naturalized host populations occur compared to sites were the host has been introduced recently (Weeds et al., 2003). Akem and Asiedu (1994) found that more than 77% of yam fields surveyed for anthracnose in Nigeria had yam grown in intercrop with an array of crops. The multitude of yam and non-yam hosts commonly found within the same field may have led to the maintenance of diverse genotypes at each location.

Weeds such as *Spigella anthelmia*, *Calapogonium mucunoides* and *Commelina* sp., and several other non-yam hosts harbour populations of *C. gloeosporioides* that can be highly virulent on yam. Cross-inoculation tests

using pathogen isolates and toxin extracts have consistently shown that *C. gloeosporioides* from yam-based cropping systems has a high cross-infection potential (Simons, 1993; Alleyne, 2001; Abang, 2003). These studies indicate that there is no justification for using the epithets f. sp. *alatae* and f. sp. *dioscoreae* suggested by Singh and Prasad (1967) and Fournet et al. (1975) within *C. gloeosporioides*. However, these tests may not accurately reflect the true infection potential because they were mostly conducted under controlled conditions in the laboratory and screenhouse using high inoculum levels and optimised humidity and temperature. These results stress the need to conduct virulence tests using whole plants in order to verify that results obtained so far are relevant in the field. Abang (2003) found that genetic differentiation among pathogen populations from different *Dioscorea* spp., mango, and citrus was low (G_{ST} = 0.10, θ = 0.034), suggesting that the same *C. gloeosporioides* population attacks both yam and non-yam hosts. This tends to support the hypothesis that alternative hosts are an important source of anthracnose inoculum.

Selectively neutral molecular markers can provide a good assessment of the population structure; however, they do not necessarily tell us anything about pathotypic variation. Eighteen *C. gloeosporioides* virulence phenotypes were identified among 217 *C. gloeosporioides* isolates using five putative *D. alata* differential cultivars but there was a weak correlation (r = 0.02, *P* = 0.40) between virulence phenotype and MP-PCR haplotype (Abang, 2003). The weak correlation between *C. gloeosporioides* pathotype and MP-PCR haplotype indicates a lack of association between genetic polymorphism and virulence. If pathotypic changes are slow, as is probably the case with the yam anthracnose pathogen, then random molecular variations will accumulate in every pathotype resulting in little correlation between pathotype and lineage (Leung et al., 1993).

The apparently high evolutionary potential of *C. gloeosporioides* on yam has important implications for anthracnose resistance breeding. The pyramiding of resistance genes has been suggested as a potentially valuable strategy in anthracnose resistance breeding in yam (Mignouna et al., 2002a, b). However, this strategy is appropriate if the pathogen is exclusively asexual and if the potential for gene flow is low. But if the pathogen is recombining and has a high potential for gene flow, as appears to be the case of *C. gloeosporioides* on yam, then the recombination of virulence alleles may occur as quickly as breeders can recombine resistance genes, thus jeopardizing all resisitance breeding efforts (McDonald and Linde, 2002a, b).

CONCLUDING REMARKS

We have attempted to show how integrating traditional and molecular approaches to understanding the systematics, epidemiology and population genetics of *C. gloeosporioides* can lead to a much better understanding of yam anthracnose disease, and thus to the development of effective and sustainable control measures. The taxonomy of *Colletotrichum* species is in flux and remains confusing. While it appears certain that FGS isolates represent *C. gloeosporioides*, it remains unclear if the SGG form should be retained at a subspecific rank within a paraphyletic *C. gloeosporioides* group species, and if the FGG form should be described as a new *Colletotrichum* species. Analysis of larger isolate samples using a combination of conventional and molecular approaches may help address these issues. Such studies will be greatly facilitated by the designation of an authentic culture to represent the name *C. gloeosporioides* - a complicated process that is still on going at CABI Bioscience, UK.

Regular sexual recombination is the most likely source of the high genetic diversity found in field populations of *C. gloeosporioides*. The sexual stage is most likely producing ascospores with the potential for wind dispersal and long distance movement. While knowledge of genetic structure may provide useful insights into the evolutionary processes that affect pathogen population genetics, experimental approaches are needed to provide a sound basis for the prediction of *C. gloeosporioides* evolutionary potential. Field experiments with marked isolates are ultimately needed to make direct estimates of rates of migration and sexual recombination in field populations. Such experiments will contribute immensely to our knowledge of the evolution of *C. gloeosporioides*, and thus to the development of sustainable management strategies for yam anthracnose disease (McDonald and Linde, 2002a).

The ability to use pathogenesis-related pathotoxins in the selection of anthracnose-resistant genotypes is expected to have a profound impact on breeding schemes for crops with long growth cycles such as yam. Yam breeding programs active in areas where particular strains or pathotypes of *C. gloeosporioides* have not been reported (e.g. India and the Caribbean) could use the toxic activity to screen pre-emptively for resistance and thus avoid the danger of international exchange of fungal cultures.

Finally, all aspects of yam anthracnose research will benefit immensely from close collaboration between IITA, which has the global mandate for yam in the Consultative Group on International Agricultural Research (CGIAR), national research partners in Africa, India and the Caribbean Islands, and advanced research institutes in the industrialised countries.

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