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POPULATION STUDIES OF FUNGAL PLANT PATHOGENS: PERSPECTIVES FOR CONTROL WITH SPECIFIC REFERENCE TO GREY LEAFSPOT

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ABSTRACT

Plant diseases hinder food production globally. Of the known crop plant pathogens, fungi are perhaps the most widely adapted organisms. For disease control, host resistance has been the main method used through major gene deployment. Equally important, has been the use of fungicides. Over the last two decades these two control strategies have been fraught with rapid resistance erosion commonly referred to as the "boom and burst cycle,". This raises urgent concerns with regard to development of effective alternative strategies such as use of fungicides. The use of fungicides is an effective strategy but they are potential sources of pollution into the environment, pose serious health risks to humans and are uneconomical for low-resource farmers. In this article the potential of how improved disease management strategies embodied in integrated disease management (IDM) can be developed based on a clear understanding of the pathosystem is discussed. We demonstrate that population and molecular genetics can be used to define pathosystems, estimate the evolutionary responsiveness of pathogens and from the data, design appropriate durable control methods. Various population and molecular genetic methodologies are described and how they can be incorporated into standard pathogen characterisation studies. Using grey leaf spot of maize *(Cercospora zeae-maydis)* as a case study, we show how these techniques can be used to generate information on genetic variability, providing for logical development of a durable IDM programme.

Key Words: *Cercospora zeae-maydis*, disease management, genetic tools, molecular markers

RÉSUMÉ

Les maladies de plante entravent globalement la production de nourriture. Des pathogènes de plante connus, les champignons sont peut être les plus repandus organismes adaptés. Pour le contrôle de maladie, la résistance hôte a été la méthode principale utilisée à travers le déploiement de gène majeur. Egalement important, a été l'usage des fongicides. Pour plus de deux décennies ces deux stratégies de contrôle ont été angoissées avec une rapide érosion de résistance communément connu à nous comme "boom and burst cycle" (cycle de boom et d'explosion). Ceci soulève les inquiétudes urgentes avec respect au développement des stratégies alternatives effectives comme l'usage des fongicides. L'usage des fongicides est une stratégie effective mais ces fongicides sont des sources potentielles de pollution dans l'environnement, pose des risques sérieux de santé aux humains et ne sont pas économiques pour les fermiers de faibles ressources. Dans cet article le potentiel de comment les stratégies améliorées de gestion des maladies incarnées dans une gestion intégrée des maladies (IDM) peut être développé basé sur une compréhension claire du pathosystème est discutée. Nous démontrons que la population et les génétiques moléculaires peuvent être utilisés pour définir les pathosystèmes, estimer les non réponses évolutionnaires des pathogènes et à partir des données, concevoir des méthodes de contrôle durables. Diverses populations et méthodologies génétiques moléculaires sont décrites et comment elles peuvent être incorporées dans les études de caractérisation de pathogène standard. Utilisant la tache grise de feuille de maïs *(Cercospora zeae-maydis)*

comme cas d'étude, nous montrons comment ces techniques peuvent être utilisées pour générer l'information sur la variabilité génétique, pourvoyant le développement logique d'un programme d'IDM durable.

Mots Clés: *Cercospora zeae-maydis*, gestion de maladie, outils génétiques, indicateur moléculaire

INTRODUCTION

Crop diseases are considered as a big threat to sustained human livelihood particularly in the developing countries whose economies largely depend on agriculture. For disease control, host resistance and pesticides have been the main strategies used. However, the success of these methods is fraught with resistance erosion and pollution from agrochemicals (Schumann, 1991). Resistance erosion is presumably due to an "arms race" between pathogens and their hosts (Stahl *et al.*, 1999; Stahl and Bishop, 2000), often manifested as "boom and burst" cycles. This event or course of events is an evolutionary response by pathogens to selection pressure caused by disease control measures (McDonald and Linde, 2002). The arms race is maintained through mutation and recombination in plant hosts and their pathogens (Meyers *et al.*, 1998; Hulbert *et al.*, 2001; Vleeshouwers *et al.*, 2001). The durability of any crop protection system will therefore depend on the use of strategies that slow-down this process.

In an effort to improve disease management, the concept of integrated disease management (IDM) was developed (Brent, 1995; Hamblin, 1995). The IDM concept attempts to employ all available disease control methods, with a goal of improving yield, a cleaner environment and slow resistance erosion (Brent, 1995; Hamblin, 1995; Agrios, 1997). Integrated Pest Management or IDM in the case of plant diseases is considered both ecological and economically sound, and is evidently the choice for disease control (Bentley *et al.*, 1995). The strong support for IDM arose from the rapid advances in plant disease epidemiology, that led to reduced pesticide use, one of the drawbacks of past disease control systems (Agrios, 1997).

Advances in plant disease epidemiology also permitted better spatial and temporal characterisation of epidemics, improving the knowledge and tools available for disease control (Zadoks and Schein, 1979). Additionally, over the past two decades, developments in molecular genetics have generated several investigative tools, leading to better understanding of host pathogen interactions. Advances in molecular genetics have also increased the versatility of population genetic tools, improving ability to study evolutionary processes in pathosystems. These developments provide the scientific framework through which improved IPM systems can now be developed by permitting the packaging of control methods that do not markedly increase evolution of novel pathogens yet remain effective (McDonald and Linde, 2002).

The durability of an IDM will nevertheless depend on the impact of disease control packages on evolutionary forces operational in pathosystems such as mutation, mating system, gene flow, genetic drift and selection. Unravelling the role(s) of these evolutionary forces in a pathosystem is embodied in population genetics.

The importance of population genetics in plant pathology today has markedly increased with recent advances in molecular genetics and bioinformatics. In this paper, the role of population and molecular genetics in the development of durable IDM strategies is reviewed. The various methodologies currently used for characterisation of pathogen populations are explored. The paper also presents using grey leaf spot of maize, a new plant disease in sub-Saharan Africa (Ward *et al.*, 1999; Okori *et al.*, 2001) as an example, a description on how population genetics is being used to define suitable disease control strategies and how this impacts on evolution of novel pathotypes, paving way for the logical use of various disease control methods in an integrated manner.

DISEASE CONTROL: A DRIVER OF EVOLUTIONARY PROCESSES IN PATHOSYSTEMS

The pathosystem. In disease management, crop diseases are viewed as integral components of stabilised, co-evolved, interacting members of an

agro-ecology, otherwise called pathosystem (Robinson, 1987). To understand the biotic interactions in a pathosystem, use of an evolutionary investigative approach is necessary. This is because the goal of most disease management approaches target manipulation of certain components of a pathosystem to better suit the crop and not the pathogen. Examples include the use of resistant varieties, crop rotation, and crop population manipulation or when pesticides are applied. These common disease control strategies increase selection pressure on pathogens resulting usually in population bottlenecks and reduction in disease spread. It is thus apparent that most disease control strategies rely on interactions between pathogens and host species, implying that the two components are the most unstable parts of any pathosystem (Robinson, 1987; Schumann, 1991; Gliessman, 1995, McDonald and Linde, 2002).

Pathosystem instability is most severe in cases where major genes have been deployed against a pathogen that has an active sexual cycle and normally manifests as resistance breakdown. Examples of recent crop epidemics in sub-Saharan Africa associated with pathosystem instability as a result of changes in the pathosystem are summarised in Table 1. In general, instability of any pathosystem is greatest when monogenic resistance has been deployed against pathogens that readily recombine such as basidiomycetes and ascomycetes. This is exacerbated under monoculture systems due to uniform crop cover over large expanses of land, creating high selection pressure on the pathogen and promoting resistance breakdown.

Modern agriculture is often characterised by monoculture systems, especially of cereals. Maize *(Zea mays)* is the most important cereal in most parts of sub-Saharan Africa, cultivated generally

TABLE 1. Examples of recent plant fungal disease epidemics in East Africa and other sub-Saharan African countries associated with disruption of equilibrium in crop agroecologies due to introduction or adoption of new technologies

Pathosystem	Possible cause of epidemics	Reference
Cercospora zeae-maydis/ Zea mays	Introduction of pathogen and no resistance in commercial varieties	Okori *et al.* (2001; 2003) Ward and Nowell (1998)
Cercospora sorghi/ Sorghum bicolor	Cultivation of susceptible hosts and incursions from wild host relatives	Mbwaga *et al.* (1993), Thomas (1991) Ngugi *et al.* (2000)
Colletotricum sublineolum/ Sorghum bicolor	Cultivation of susceptible hosts and incursions from wild host relatives	Mbwaga *et al.* (1993) Ngugi *et al.*(2000), Marley *et al.* (2001)
Exserohilum turcicum/ Zea mays	Introduction of susceptible varieties and wide scale adoption causing rapid inoculum build-up	Adipala *et al.* (1993), Borchardt *et al.* (1997)
Phytopthora infestans/ Solanum tuberosum	Large-scale use of metalazyl a systemic fungicide	Mukalazi *et al.* (2001)
Mycosphaerella fijiensis/ Musa spp.	Introduction of pathogen and no resistance in banana clones	Tushemeriewe and Waller (1993), Mouliom *et al.* (1996)
Fusarium xylairoides/ Coffea coffeanum	Introduction of pathogen and no resistance in commercial clones and cultivars	Lukwago and Birikunzira (1997)
Fusarium oxysporum f.sp. cubense) *Musa* spp.	Exotic bananas more susceptible than East African highland bananas	Kangire (1998)

as a monocrop with new variety deployment being quite common. It therefore provides an excellent case for rapid resistance breakdown as appears to be the case with turcicum leaf blight (*Exserohilum turcicum*), whose epidemics are frequent in sub-Saharan Africa (Adipala *et al.*, 1993; Borchardt *et al.*, 1997). For maize and other crop species, elucidation of evolutionary processes in a pathosystem could markedly improve disease management by defining evolutionary dynamics within pathogen populations. Population genetics would also provide explanation(s) for evolutionary processes by addressing strategic questions related to pathosystems (Leung *et al.*, 1993; McDonald, 1997; Milgroom and Fry, 1997). Such questions include the following: How large the pathogen population is? What constitutes an epidemiological unit? What amount(s) of genetic variation exists in the population? What are the causes of the genetic variation in the population? What control measures can be put in place to minimise rapid evolution of the pathogen? and What is/are the anticipated response(s) to selection by the pathogen. The answers to these questions have in fact been the basis for IDM development over the last two decades and the methodologies that have been developed to estimate genetic variation are discussed below.

The use of population genetics in development of an IDM strategy. Population genetics is a field concerned with determining the extent and pattern of genetic variation in populations with a goal of understanding evolutionary processes affecting origin and maintenance of genetic variation (Milgroom and Fry, 1997). It is therefore a core part of evolutionary biology. It is necessary however, to make a clear difference between systematics and population genetics, which are components of evolutionary biology. Systematics focuses on drawing phylogenetic inferences between species in the same or higher taxa based on morphological or genetic data. Population genetics on the other hand, utilises information on genetic variation (gene and genotype frequencies) to elucidate the role(s) of evolutionary processes such as gene flow, genetic drift, selection, mutation and mating systems usually within a species at a micro-evolutionary scale. Because population genetics like systematics or even the need to design diagnostic tools are dependent on genetic variability, it is in many cases confused with those disciplines (Milgroom and Fry, 1997).

A common case in plant pathology is when phenetics or phylogeny is used to distinguish among members of a pathogen species or even pathotypes and confused with population genetics. On the contrary, in the above example, population genetics would focus on elucidating the evolutionary cause(s) of genetic variation between and among the pathogen population. It is worth mentioning that the use of systematics and population genetics in plant pathology can be mutually reinforcing when the merits of each study approach are well utilised.

Methods for detecting variation in pathogen populations within pathosystems. It is important to note that genetic variability of any organism is directly linked to its recent evolutionary history and reproductive biology. These two core attributes of an organism directly relate to its evolutionary potential. Evolutionary potential, in turn, is directly proportional to the amount of genetic variation in a population (Fisher, 1930). Genetic variation of a population can be measured on the basis of either ecologically important traits or selectively neutral genetic markers. Variation based on ecologically important traits has direct meaning in disease management since prediction on response(s) to selection by pathogen populations can be made directly. Ecologically selective traits have been used for a long time to characterise pathogen populations. This is based on the fact that implementation of any control method increases selection pressure on a pathogen, and consequently, studying genetic variability using phenotypes subject to selection could predict durability of a disease management practice. An example of a commonly used ecologically selective trait to characterise fungal pathogen populations is fungicide sensitivity (Kadish and Cohen, 1988; Day and Shattock, 1997; Romero and Sutton, 1998; Mukalazi *et al.*, 2001). Such a trait is directly affected by selection and is reliable when integrated with epidemiological investigations (Wolfe and McDermont, 1994; Brown, 1995). Unfortunately, the number of

samples that can be studied using ecologically selective traits is usually small, limiting their scope for population studies.

The use of race differentials is another method that could be regarded as dependent on ecologically selective traits because it is based on differences in pathogenicity among pathotypes. But like other ecologically selective traits, it too is constrained by the number of samples that can be handled at once. Pathotype analysis based on race differentials may also be influenced by the environment, is subject to judgemental biases of the investigator and often, unknown resistance factors can compound analysis. The most serious disadvantage of relying entirely on ecologically selective traits to infer population variability is that the genes involved in a particular host-pathogen interaction only represent a small fraction of genes in that pathogen population and may thus not represent the genetic diversity of the whole population. Within the same race, there may also be variations at molecular level that may escape detection. An example is the case of *E. turcicum* in East Africa where Adipala *et al.* (1993) using race differentials identified only race O of the pathogen. Four years later, Borchandt *et al.* (1997) using neutral genetic markers reported extensive variation among and between East African *E. turcicum* populations. This shows that ecologically selective traits though useful, clearly have some limitations. Their limitations in estimation of genetic variation among and between pathogen populations will largely be determined by factors relating to the pathogen and ease of the estimation procedure. Genetic analyses based on selective traits can be improved using other techniques such as those based on selectively neutral genetic markers.

Selectively neutral genetic markers unlike the ecologically important traits are not related to the fitness of the pathogen. In general, the use of neutral genetic markers in the study of plant pathogen populations has dramatically improved our understanding of pathosystems. There are several reasons as to why neutral genetic markers are widely used today. These include; ease of use, more samples can be handled per unit time, analyses can be easily combined with other techniques, when linked to gene loci of interest, they can be used in marker assisted selection and are amenable to recent advances in genomics and bioinformatics (McDonald and Linde, 2002). Neutral genetic markers are found largely in non-coding regions of the genome that are less conserved and prone to mutations and are also less conservative, hence highly variable (Michelmore and Hulbert, 1987). Inference of phylogeny and other population genetic characteristics of a pathogen rely on presence of polymorphism that relates to evolutionary processes in a species. Neutral genetic markers satisfy these requirements and are thus widely used for these purposes.

Today there are a number of genome characterisation techniques based on selectively neutral DNA-based genetic markers. Commonly used techniques include, random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995), restriction fragment length polymorphism (RFLP) and inter simple sequence repeats (ISSR) (Pradeed-Reedy *et al.*, 2002). The RFLP technique may also involve DNA finger printing based on probing of DNA with genomic clones. It may also involve restriction analysis of PCR products of nuclear and mitochondrial rDNA genes and intergenic regions (White *et al.*, 1990; Bruns *et al.*, 1991; 1992). Use of DNA finger printing is most appropriate for detection of clonal lineages and is a useful tool, especially in asexually reproducing fungi as has been shown in several fungi (Boerger *et al.*, 1993; Kohli *et al.*, 1995; Kumar *et al.*, 1999; Rosewich *et al.*, 1999a,b). We have used DNA finger printing to detect *Cercospora zeae-maydis* clones of similar clonal lineage in East Africa populations (Fig. 1). DNA finger printing could also be done using AFLP or ISSR. The ISSR products are resolved by electrophoresis on 1.5% agarose gels and are thus one of the cheap DNA finger printing techniques.

Genetic variation can also be investigated by sequencing of specific genes and performing analyses for variation. For plant pathogens, the commonly sequenced regions are the internal transcribed spacer (ITS1 5.8S ribosomal ITS2) ribosomal DNA (rDNA), mitochondrial small sub-unit rDNA genes (mtSSU) (White *et al.*, 1990), β- tubulin gene (Skovgaard *et al.*, 2001), translation elongation factor 1α (EF-1α) coding

region and introns (O'Donnell *et al.*, 1998) among others. Sequencing of DNA/genes directly linked to a trait such as resistance could also be done and used in analyses. Of all the above listed genes, the ITS region has been the most frequently used. Most ITS sequences have been deposited with public databases such as EMBL or GeneBank for comparative phylogenetic analyses and are available from their websites. Analysis of variation from a gene is done on the basis of variation in mutation of DNA that may result from nucleotide substitution, insertion, deletion or inversion. This level of analysis of variation is more precise and provides for wide taxonomic description of plant pathogens. It can also generate data that show sources of pathogens and gene flow between regions. It is however, expensive and requires good working knowledge of phylogenetics.

Electrophoretic karyotyping using pulsed field gel electrophoresis (PFGE) is another useful genomic tool, especially for linkage analysis and genome characterisation (Mills and McCluskey, 1990; Zolan, 1995). The PFGE involves migration of intact chromosome-size DNA on which an electric pulse field is applied in an agarose gel. Southern hybridisation with a known gene will allow identification of linkage groups. Data from PFGE when combined with flow cytometry, which estimates genome size, improves further characterisation of genomes (Lu, 1996).

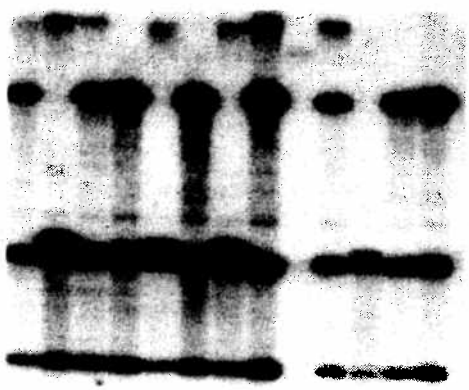

Figure 1 A

Figure 1 B

Figure 1. Restriction fragment length polymorphic fingerprints of *Cercospora zeae-maydis* isolates from Uganda. Isolates with the same fingerprint were considered as clones. Clone identity is clear from the upper and lower panels obtained from two independent RFLP analyses. The two filters were made from total genomic DNA digested with either *Hind*III and probed using genomic clone 28 (A) or *Eco*RI and probed using genomic clone 175 or digested using (B). The probes used and the entire RFLP process were performed according to Okori *et al.* (2004).

Neutral genetic markers as substitutes for ecologically important traits: a caution. The ease of experimentation and relatively low costs makes neutral genetic markers attractive as population genetics investigative tools (Milgoom and Fry, 1997). The caution, however, is that prediction of ecologically important variation on the basis of selectively neutral genetic markers may not be correlated. This is due to variation in mutation rates and selection pressure at selectively neutral and ecologically important loci. Examples of fungi where genomic polymorphism based on neutral genetic markers is not correlated with pathotype data include *Magnapothe grisea*, the rice blast fungus (Zeigler *et al.*, 1995) and the wilt fungus, *Fusarium oxysporium* (Appel and Gordon, 1994). The above examples are ascomycete and deuteromycete fungi, respectively, and similar phenomena may occur in other fungal orders and species.

Neutral genetic markers may nevertheless be used as predictors of ecologically important traits in cases where there is evidence of tight linkage between the two loci (Brändle *et al.*, 1997). Linkage is attributed to gametic disequilibria, especially in obligate asexually reproducing fungi (Brändle *et al.*, 1997; Milgroom and Fry, 1997). Under such circumstances, analysis of data based on neutral genetic markers must be interpreted alongside genetic linkage information as has been done for the powdery mildew fungus, *Erysiphe graminis f.sp. hordei* (Wolfe and McDermont, 1994; Brändle *et al.*, 1997). Nevertheless, selectively neutral genetic markers provide good insights to questions related to key evolutionary processes such as gene flow, mating systems and population size than ecologically important traits, as has been demonstrated in a number of fungal pathogens (Kohli *et al.*, 1995; Brändle *et al.*, 1997; Kumar *et al.*, 1999; Rosewich *et al.*, 1999a,b; Samils *et al.*, 2000; Okori *et al.* 2003).

Selectively neutral genetic markers can also be used for monitoring dispersal of identified pathogen genotypes using known markers. Pathogen tracking as such a process would be referred to, has potential use in epidemiological studies, particularly relating to pathogen dispersibility (Zhan *et al.*, 2001). Detailed description of techniques based on neutral genetic markers, their appropriateness and or weakness are extensively reviewed elsewhere (Kohn, 1992; Leung *et al.*, 1993; McDonald and McDermont, 1994; McDonald, 1997; Milgroom and Fry, 1997). It should however, be emphasised that an understanding of an ecologically important variation is needed in order to predict the durability of any management practice. It therefore follows, that for disease management, these new advances in molecular biology and population genetics are supplementary tools for refinement of existing pest management system study methodologies.

Statistical considerations for population genetic analysis. Statistical inference on any data set can only be meaningful if tenets of the analysis are upheld. In population genetics, the starting point is to sample the data well enough to capture genetic variation in a given population. Since most population genetic analyses are based on allele frequencies at separate loci, the samples must therefore be structured such that correct inferences can be made about the population. The correct sample size however, depends on ones definition of a population. It is important to clearly understand what constitutes a population and how large the sample size should be. Fungal pathogens may not satisfy the ecological definition of a population that restricts a population to members that can inter-breed. The definition by Krebs (1985), whereby, a population is considered as a group of organisms of the same species occupying a particular space at a particular time is appropriate for fungal pathogen populations.

Sample size depends on the biological question being investigated and type of pathogen, whether aerial or soil borne. In general, estimates of allele frequencies based on sample sizes of 3-10 individuals per population are usually meaningless. Whereas estimates based on sample sizes of 30-100 individuals can be quite reliable. Questions relating to genetic distance may be adequately addressed with a sample size of 30-40 individuals per population whereas those relating to selection coefficients require sample sizes of hundreds or even thousands (McDonald, 1997). Sample size also depends on analytical tools to be used (Lynch and Milligan, 1994; Weir, 1996).

Neutral markers can be used as reliable analytical tools. Selectively neutral markers are classified as dominant or co-dominant depending on ability

to detect heterozygosity. The RAPD and AFLP are examples of dominant markers, while the RFLPs are co-dominant. Dominant markers have a two-allele system (present or absent), which greatly limits their use in genetic diversity affected by the number of alleles at a locus (McDonald, 1997). Dominant markers also have different sampling properties from co-dominant ones and this must be considered in the experimental design (Lynch and Milligan, 1994: Jorde *et al.*, 1999). Gene frequency estimates from dominant markers are generally less accurate than those obtained from co-dominant markers (Jorde *et al.*, 1999). The most practical way to improve the accuracy of estimates of genome-wide parameters such as average gene diversity, when dominant markers are used is to use large sample sizes (about 100 individuals) per population (Lynch and Milligan, 1994). Dominant markers are however useful and reliable especially in asexually reproducing fungi or fungi whose population structure is composed of clonal lineages and can be optimised for better analysis of population structure by increasing sample size by up to 20 times that required for co-dominant markers (Lynch and Milligan, 1994). For most purposes, hierarchical sampling is the best starting option, especially if no prior knowledge of population structure is known. Sampling must therefore be made in such a way that genetic attributes of the population at all levels of hierarchy are captured. The various statistical approaches, beginning by focusing on population genetic analyses and phylogenetics.

The analytical tools. The goal of performing population genetic analyses is to provide a basis for inference of evolutionary processes in natural pathogen or pest populations (Weir, 1996). Elucidation of population structure is the starting point for this purpose, since it permits logical prediction of evolutionary responses of the pathogen population in question. Population structure refers to the extent to which large populations are sub-divided into smaller sub-populations that may differ in allele frequency from the neighbouring sub-population. Population structure is a useful indicator of the amount of genetic variation between and within populations. It should however, be noted that since population structure is obtained by partitioning of genetic variation, inference of population structure is highly dependent upon the tools used to estimate genetic diversity. Different types of markers can yield different relationships among isolates from the same population (Leung *et al.*, 1993). In general, we gain more insights with techniques that have higher discriminating power. One of the consequences of population structure on an organism's population is the reduction of heterozygous individuals relative to the average proportion of heterozygous genotypes expected under random mating. Wright (1951), developed statistics for estimating reduction in heterozygosity or inbreeding effect of population substructure at each population hierarchy, otherwise called Wright's F statistics or fixation index. Wright's F statistics equals the reduction in heterozygosity expected with random mating at any level of a population hierarchy relative to another species more inclusive level of the hierarchy. The genetic symbol for the fixation index is F embelished with subscripts denoting levels of hierarchy being compared.

For questions relating to population structure, Wright's F statistics can be estimated directly from genetic data or by analysis of molecular variation (AMOVA) (Excoffier *et al.*, 1992). Analysis of molecular variation is appropriate for most neutral genetic markers whether dominant or co-dominant. Other analyses include estimation of gene diversity or average heterozygosity at discrete loci (Nei, 1987).

Genotype diversity is another informative parameter of the genetic structure within populations. In species reproducing both sexually and asexually, genotype diversity can be used to estimate the relative extent of clonal contribution within a population, or between populations (Stoddart and Taylor, 1988). Thus both genotype diversity and gene diversity are indeed appropriate for measuring variability, especially among asexually inbred populations (Weir, 1996; McDonald, 1997). The majority of phytopathogenic fungi are either ascomycetes or deuteromycetes (Pollack, 1988; Goodwin *et al.*, 2001). These fungi, mainly asexually form epidemiological populations (Burdon, 1993) and therefore, the use of gene diversity and genotype

diversity is advisable for these pathogens. Additionally, on the basis of population structure, indirect or direct estimates of gene flow between populations can be calculated (Slatkin and Barton 1989; McDermont and McDonald, 1993). Gene flow can occur either by spore movement as in the case of long distance transport of asexual or sexual spores or by movement of individuals between different populations. Movement of individuals is generally more dangerous in terms of influencing population structure and epidemics than spore movement. This is because the exchange of individuals between two populations is equal to movement of selectively adapted alleles into new environments. This type of gene flow is also referred to as genotype flow and has been the major cause of some of the most severe plant disease epidemics (McDonald and Linde, 2002). Gene flow estimates are otherwise helpful in defining geographical boundaries, an important aspect in disease management.

Furthermore, depending on the research question, data may also be subjected to molecular phylogeny. Molecular phylogeny refers to the study of evolutionary relationships among organisms. Inferring phylogeny is however, an estimation procedure of the evolutionary history based on incomplete information contained in a given data set (Swafford *et al.*, 1996). This is because we generally do not have direct information about the past but instead have only access to contemporary species or molecules. Nevertheless, molecular phylogeny can be used to resolve questions related to population structure, analysis of mating systems, gene evolution, individual relatedness, species boundaries and hybridisation (Hills *et al.*, 1996). Phylogeny can be estimated using distance-based or character-based methods. Distance-based methods use the amount of dissimilarity between data sets to derive trees.

In general, distance-based methods would reconstruct the right evolutionary tree if all genetic divergence events were accurately recorded in the sequence (Swofford *et al.*, 1996). Distance-based methods compute pair-wise distances or quantitative comparisons between two species or sequences. In the process, data from characters are discarded and only genetic distances used to derive trees. For example, different sequences may generate the same distance matrix, and given only the distance matrix, it is impossible to go back to the original sequences. The accuracy of distance-based methods is further constrained as divergence encounters an upper limit when sequences become mutationally saturated reducing the likelihood of inferring realistic evolutionary phylogeny (Brinkman and Leipe, 2001). Examples of distance-based methods include, neighbour joining (NJ), the unweighted pair-group method with arithmetic average (UPGMA) and methods that optimise additivity of distance trees such as minimum evolution (ME). Character-based methods derive trees that optimise the distribution of the actual data patterns for each sequence and tend to be computationally demanding. Tree distances are not fixed as they are determined by each tree topology. Examples of character-based methods include maximum parsimony and maximum likelihood. Swofford *et al.* (1996) and Brinkman and Leipe (2001) provide excellent reviews of available methods. Sequences may also be analysed for base-pair changes and in that case, older populations with existing population structure will have accumulated more mutations and thus more base-pair differences. It is worth noting that most of the population genetic analyses mentioned in this article and many more can be performed by free software. A good starting point is Dr. Joe Felsenstein's home page http://evolution.genetics.washington.edu/phylip/software.html. As emerging discipline in plant pathology, population genetics and molecular phylogeny open frontiers for deeper understanding of pathosystems, by providing a new generation of statistical and evolutionary tools that strengthen ecologically based studies for the development of sustainable pest management systems.

GENETIC STUDIES OF EAST AFRICAN *Cercospora zeae-maydis* POPULATIONS

Grey leaf spot of maize caused by *Cercospora zeae-maydis* Tehon and Daniels is one of the most important foliar diseases of the crop in both temperate and tropical ecologies, causing yield losses of up to 60% in susceptible genotypes (Ward *et al.*, 1999). The disease has only been recently reported in East Africa (Pratt *et al.*, 1997;

Okori *et al.*, 2001; Asea *et al.*, 2002). Farmers in East Africa are resource poor and therefore disease management strategies should be affordable and sustainable (Nelson *et al.*, 2001). Integrated disease management has been suggested as economically viable even for resource-poor farmers (Bentley *et al.*, 1995; Nelson *et al.*, 2001). Being a new disease in East Africa, a number of questions arose; Are East African *C. zeae-maydis* populations introduced from the US or elsewhere? What is their population structure? What could be the most prominent evolutionary force operative in the populations? What management methods could be used to slow down disease spread and novel pathotype evolution? We undertook a number of studies with the overall goal of gaining information on genetic variability of the pathogen and its evolutionary responsiveness to control methods as a first step towards development of an IDM for grey leaf spot. A hierarchical sampling strategy was used to collect isolates from Uganda, Kenya, Rwanda and in addition, samples were drawn from Zimbabwe (Fig. 2) and for comparative purposes, US isolates were included. The American isolates had been characterised using molecular methods and reportedly belonged to two pathotypes designated group I and II (Wang *et al.*, 1998). The neutral genetic markers, AFLP and RFLP were used to investigate genetic diversity and to study the role of gene flow and genetic drift in distribution of genotypes within and among populations (Okori *et al.*, 2003). Data from these studies estimated genetic variability, identified operative evolutionary forces and the size of *C. zeae-maydis* epidemiological units. Molecular data were subjected to population genetic analyses of AMOVA, estimates of gene diversity, gene flow and phylogeny.

No population structure was detected among African *C. zeae-maydis* populations, implying that genetic similarity between geographically isolated East African populations. Comparisons with US isolates revealed similarity between East African and US group II pathotype, while AMOVA revealed presence of a moderate population structure between African and US

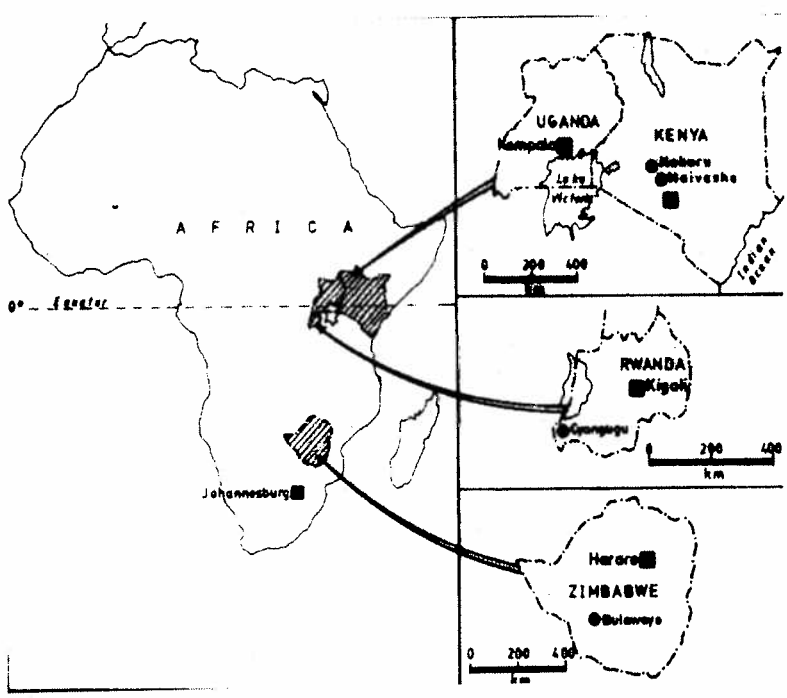

Figure 2. Map of Africa showing various sampling sites from which *Cercospora zeae-maydis* isolates were collected as part of a population genetic study of the pathogen. A hierarchical sampling strategy was used at all sites

isolates ($\Phi F_{ST} = 0.17$). Phenetic analysis detected only one pathotype (group II) in East African populations. Gene diversity estimates were higher among East African populations compared to US group I pathotype, but were comparable with US group II isolates. The gene flow estimator Nm was high (4.75). Slatkin and Barton (1989) have shown that Nm values greater than 1 imply that gene flow is strong enough to prevent substantial differentiation due to genetic drift. Supportive evidence for gene flow in East Africa comes from recent epidemiological studies in Uganda, which indicated aerial transport of spores and easy spread of propagules from infected foci (Asea *et al.*, 2002). Overall, these results showed that East Africa is one epidemiological unit with regard to grey leaf spot, irrespective of the geographic distance between the East African countries. Moreover, the high genetic similarity among East African isolates also indicates that the population is largely clonal and that, a sexual phase may be rare or non-existent. Although, genetic relatedness to US group II pathotype is perhaps indicative of common recent ancestry from an evolutionary point of view, the East African *C. zeae-maydis* populations would be considered as having low evolutionary responsiveness. However, due to the large epidemiological units among the East African *C. Zeae-maydis* the probability for accumulation of new variant genotypes cannot be overly underestimated.

IDM for grey leaf spot development based on population genetic studies. Our data from population genetic analyses provide important information for making decisions for grey leaf spot control in Uganda and the region in general. The data describe genetic variation within and between East African *C. zeae-maydis* populations and provides for estimation of gene flow and clonal attributes of the pathogen. The data also indicate the evolutionary potential or risk of *C. zeae-maydis*. McDonald and Linde (2002) have developed a system through which data from population genetics and pathogen biology can be used to predict the evolutionary risk associated with a plant pathogen. According to the scheme, presence of a sexual phase and or large effective population size of a pathogen increases the evolutionary risk. In the case of *C. zeae-maydis*, the risk would be rated as low to medium, due to its inherent anamorphic state and occurrence of gene flow in East Africa. As a consequence, components of an IDM targeting inoculum production, survival and efficiency should be able to reduce grey leaf spot epidemics in the region and should be used across national frontiers since the region is one epidemiological unit. A primary source of inoculum production and survival is the practice of continuous cropping of maize in most parts of East Africa. Host resistance has been shown to be effective in curtailing sporulation (Freppon *et al.*, 1994), while deep ploughing is effective in reducing initial inoculum (Beckman and Payne, 1982). For most farmers, in the sub-Saharan Africa, mixed cropping and long rotation cycles are evident cultivation practices that could be used to control inoculum build-up. The caution however, is that even fungi that are known to be "obligate anamorphs" retain some means of recombination (Leslie and Klein, 1996; Brändle, 1997). Our data showed high gene diversity among East African *C. zeae-maydis*, indicative of some genetic variability. *Cercospora zeae-maydis* is a deuteromycete and as an "obligate anamorph," it may posses other means of recombination. Use of fungicides that target single genes or more commonly, deployment of major resistance genes is clearly risky due to extensive gene flow within the region and the varied agroecologies that may support novel pathotype evolution. Thus, a diligent rotation of control packages is worthwhile to apply to stem selection of virulent clones and consequent disease explosion. Breeding for resistance should be done at regional level to offset gene flow effects and the maximise use of scarce resources. The success stories for grey leaf spot control will however, require concerted efforts over wide areas and combinations rather than single management options.

CONCLUSIONS AND FUTURE PERSPECTIVES

Increasing emphasis is being placed on IDM as the strategy most suited for control of diseases in sustainable crop management systems. The success of IDM in agricultural production systems will however depend on usage of crop management

practices in the right balance. The goal of such management options should be to curtail resistance erosion by balanced use of practices that do not excessively disrupt pathosystems. Excessive disruption of pathosystems increase selection pressure and evolution of novel pathogen genotypes. This is a process one would like to avoid for the long-term success of an IDM strategy. To achieve this, integrated use of epidemiology and population genetics is necessary. Plant disease epidemics result from interaction between components of a pathosystem or disease pyramid. Conducting epidemiological studies will reveal the rate determining components of epiphytotics, which control methods should target. While population studies will elucidate evolutionary potential of the pathogen. This combined application of epidemiology and population genetics has been successfully used in North America to control rust of wheat (Agrios, 1997). For sub-Saharan Africa, the challenge today is how to integrate data being generated from population genetics and epidemiological studies of various pathogens in order to design effective IDMs for various diseases. For most diseases in sub-Saharan Africa, there is paucity of information on pathogen populations when compared to that available elsewhere. The challenge therefore is to begin to conduct some of these studies in the region. In this way, it will be possible to link disease management protocals to well designed control strategies based on a wealth of information generated locally. Also importantly, though IDM is suggested as being more holistic in design and application, very little is known of pathosystem interactions that may favour evolution of novel pathotypes under such management. There are very few studies on selection under different farming systems and consequently, lack of much information needed to formulate realistic hypotheses about pathotype evolution and more importantly, resistance breakdown (Ennos and McConnell, 1995). This is an area of research that plant pathologists need to venture into if we are to strengthen the fight against plant pathogens, once described as "treacherous enemies" in the 1940's (Stakman, 1947). Overall we consider that integration of population studies in the design of crop disease management systems is absolutely necessary in order to improve the durability of disease control methodologies. This is essential in both asexual and sexually propagated fungi for the long-term success of any IDM.

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