African Crop Science Journal, Vol. 23, No. 1, pp. 45 - 57
ISSN 1021-9730/2015 $4.00

DYNAMICS OF *Cercospora zeina* POPULATIONS IN MAIZE-BASED AGRO-ECOLOGIES OF UGANDA

P. OKORI, P.R. RUBAIHAYO, E. ADIPALA[1], J. FAHIESON[2] and C. DIXELIUS[2]
Department of Agricultural Production, Makerere University, P. O. Box 7062, Kampala, Uganda
[1]Regional Universities Forum for Capacity Building in Agriculture, Makerere University, P. O. Box 7062, Kampala, Uganda
[2]Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, P. O. Box 7080, S-750 07 Uppsala, Sweden
Corresponding author: P.Okori@cgiar.org, P.Okori@caes.mak.ac.ug

(Received 3 March, 2014; accepted 20 December, 2014)

ABSTRACT

Stability of pathogen populations characterised by slow temporal variation is important for durability of disease management systems in any agroecology. Temporal variation in population structure is attributed to factors related to ecology, biology and life history, and varies among organisms and ecosystems. The objective of this study was to investigate genetic variability of *Cercospora zeina* (previously called *Cercospora zeae-maydis* Type II) populations in maize (*Zea mays*) producing areas under Uganda conditions. Populations of the fungus were analysed for genetic variability using a fluorescent amplified fragment length polymorphism (AFLP) technique. Little or no genetic differentiation (ϕF_{ST} 0.05) was detected for populations sampled within the same year, within an agroecology. However, a weak to moderate population structure was detected between populations from different locations, within the same (ϕF_{ST} = 0.08) or different agroecologies (ϕF_{ST} = 0.09). Pair-wise comparisons using ϕF_{ST} gene diversity and genetic distance, showed a reduction in genetic diversity in younger populations, suggestive of minor effects of selection and genetic drift. Overall, the data suggest that during the 3 years of study the impact of selection and genetic drift on *C. zeina* populations in the two Ugandan agroecologies is slow, but progressive leading to homogenetity with agroecologies and differences between agroecologies.

Key Words: *Cercospora zeae-maydis, Cercospora zeina*, genetic, structure, *Zea mays*

RÉSUMÉ

La stabilité des populations d'agents pathogènes caractérisée par une faible variation dans le temps, est importante pour la durabilité dans les systèmes de gestion des pathologies des plantes dans n'importe quelle zone agro-écologique. La variation dans le temps au sein d'une population, est fonction de facteurs relatifs à l'écologie, la biologie et l'histoire de vie des pathogènes. Elle varie d'un être vivant à un autre et d'un écosystème à un autre. L'objectif de cette étude était d'évaluer la variabilité génétique au sein des populations de *Cercospora zeina* (précedemment appelé *Cercospora zeae-maydis* Type II) dans les zones productrice de maïs (*Zea mays*) en Ouganda. Les populations de ce champignon microscopique ont été soumises à une étude de variabilité génétique grâce à la technique du polymorphisme de longueur de fragments amplifiés (AFLP). Très peu ou aucune variation génétique (ϕF_{ST} 0.05) n'a été observée pour les populations échantillonnées au cours de la même année, dans une zone agro-écologique donnée. Néanmoins, une structure populationnelle d'envergure faible à modérée a été observée entre les populations de différentes origines, (ϕF_{ST} = 0.08) à l'intérieur d'une même population ou (ϕF_{ST} = 0.09) entre les populations de différente zones agro écologique. La comparaison par paires utilisant ϕF_{ST} diversité des gènes et distance génétique, a montré une réduction de diversité génétique dans les populations les plus jeunes, suggérant ainsi un effet mineur de sélection et de dérive génétique. Au total, les données collectées indiquent un faible impact de sélection et de dérive génétique sur les populations de *C. zeina* dans les deux zones

agro-écologiques Ougandaise durant les 3 années de l'étude, mais cet impact est progressif et responsable de l'homogénéité au sein des zones agro-écologiques et des différences entre les zones agro-écologiques.

Mots Clés: *Cercospora zeae-maydis, Cercospora zeina*, génétique, structure, *Zea mays*

INTRODUCTION

Agro-ecosystems are purposely-altered natural ecosystems for agricultural production. In natural and agro-ecosystems, there is often evidence of sub-systems involving pathogens and their hosts called pathosystems (Robinson, 1987; Agrios, 2005). These pathosystems, account for disease outbreaks reported in natural and agro-ecosystems, with more outbreaks that can be widespread occurring in agro-ecosystems. Increased frequency of epidemics in agro-ecosystems is in part, due to destabilisation of ecosystem equilibria, often by deployment of uniform crop genotypes that invariably create high selection on pathogens, enhancing microevolution of novel pathogens (Gliessman, 1995).

Most epidemics are caused by pathogen populations that are usually constituted by asexual propagules such as conidia (Burdon, 1999; Milgroom and Peever, 2003). These asexually produced propagules contain selectively adapted pathogenicity alleles for crop varieties that they have been exposed to, and as such, become ineffective when new host plant resistant alleles are deployed against them. Thus, humans invariably, through crop breeding, influence patho-system stability by altering the intensity of micro-evolutionary processes. This could, in the long run, affect crop productivity, which is the focus of modern agriculture, especially for crops such as maize (*Zea mays* L.) that are distinguished by multiple disease infections annually.

Maize is cultivated in warm tropical and sub-tropical areas, conditions suitable for fungal pathogens. Worldover, the threat posed by leaf blights and spots, remains high, with grey leaf spot (*Cercospora zeae-maydis* Tehon and Daniels) being a major threat. *Cercospora zeae-maydis* is a necrotroph that survives on infested maize debris (de Nazareno *et al*., 1993; Asea *et al.,* 2002). The pathogen is generally a weak competitor with no known sexual phase (Chupp, 1955; Ward *et al*., 1999). The pathogen has two species associated with epidemics worldwide (Wang *et al*., 1998; Okori *et al*., 2003).

In Africa, the genetically diverse type II sibling species, reclassified as *Cercospora zeina,* is the most predominant (Dunkle and Levy, 2000; Okori *et al*., 2003; Crous *et al*., 2006). *Cercospora zeina* is the most common pathotype in Africa, with no reported cases of *Cercospora zeae maydis* made so far (Meise *et al*., 2009).

In East Africa, the disease was first reported in the late 1990s; since then, the disease is endemic in most of sub-Saharan Africa (Pratt *et al*., 1997; Bigirwa *et al*., 1999; Berger *et al.,* 2014). Over the years, resistant genotypes have been deployed to manage the disease, invariably influencing pathogen population dynamics in the *C. zeina,* maize pathosystem (Pratt and Gordon, 2006). Indeed, grey leaf spot epidemics, have remained erratic with resurgence being quite common. A study of micro-evolutionary processes in populations of *C. zeina* would shed some light on what is happening.

This study on the population dynamics of *C. zeina* was initiated almost seven years after the first reporting of grey leaf spot in East Africa. Other studies had shown that the East African *C. zeina* populations had no genetic structure, with most variation attributed to within population rather than between population differences (Okori *et al*., 2003). The pathogen also regularly undergoes microcycle microconidiation, a process through which the fungus produces spores, without an intervening phase of vegetative growth (Lapaire and Dunkle, 2003). Microconidiation is associated with exposure to stress (Lapaire and Dunkle, 2003). The outcome of the deployment of resistant genotypes and management in the east African *C. zeina* pathosystem is unknown.

Studies elsewhere reported temporal dynamics in population structure of pathogens and the associated epidemics (Lamour and Hausbeck, 2001; Zhan *et al*., 2001; Gomes *et al.,* 2003). Elucidating the temporal dynamics in *C.*

zeina populations would permit inference on evolutionary responsiveness of the pathogen to guide disease management (Provine, 1986; Lederberg, 2000; McDonald and Linde, 2002).

The objective of this study was to investigate changes in population structure of *C. zeina* in Uganda.

MATERIALS AND METHODS

Study site. Infected leaves were collected from three maize growing districts of Uganda, namely, Kapchorwa, Masaka and Wakiso. These districts belong to two main agroecological zones, that is, highland and Lake Victoria Crescent. The two agroecologies were selected because of differences in climate and farming systems. Kapchorwa district is located in the highland agroecology, at over 1400 m above sea level, with a mean temperature of 20 °C and annual rainfall of 1200 mm received during one long rainy season (Wortman and Eledu, 1999). In Kapchorwa, maize is usually produced from hybrids. The use of grey leaf spot resistant hybrids in Kapchorwa started in 2002.

The second agroecology, the Lake Victoria Crescent, was represented by Masaka and Wakiso districts. Lake Victoria Crescent is on average 1174 m above sea level, with mean temperature of 20 °C and mean annual rainfall of 1200 mm spread over two rain reasons (Wortman and Eledu, 1999). In the Lake Victoria Crescent, farmers mainly use open-pollinated maize varieties. In Uganda, improved maize genotypes are either moderately resistant (such as Longe-1, an open pollinated variety) or highly resistant such as the hybrid SC 627 (Bigirwa *et al.,* 1999; Okori *et al*., 2004a). In both study sites, farmers who consistently planted maize in a 2 Km radius were identified and their fields were used in initial and subsequent assessments for over a three year period. These farmers had, in general, planted improved moderately resistant or resistant hybrid varieties. The 2 Km radius was selected to permit collection of representative sample sizes because of the small farm sizes (usually less than 1 ha).

Sampling was done along a transect at 20 m intervals, taking 3-4 leaf samples per site, from 5 to 10 different farms. Previous studies showed that at over 10 m from the inoculum source, disease severity significantly decreases and spread is attributed to aerial inoculum (de Nazareno *et al*., 1993; Ward *et al.,* 1999; Asea *et al.,* 2002). A distance of 10 m was added to minimise sampling of the same pathogen clones.

The samples collected and used in the study from the highland agroecology were 64 and 116 collected in 2002 and 2003; and from Lake Victoria Crescent, Masaka, 63, 55, 52, 101, and Wakiso, 44, 64, 93 collected in 2001, 2002 and 2003, respectively. Leaf samples were air-dried, single spore cultures prepared and DNA isolated (Dunkle and Levy, 2000; Okori *et al.,* 2003).

AFLP analysis. Genomic DNA was subjected to AFLP analysis based on a fluorescent labelling technique as described (Okori *et al*., 2003; 2004b). All primers used in different steps are listed in Table 1. Selective amplification was done using the MseI primer Pre-MseI C, togetherwith the three EcoRl selective primers; giving a total of three primer combinations. The EcoRI selective

TABLE 1. Description of primers used for amplified fragment length polymorphism (AFLP) analysis for *Cercospora zeae-maydi* in Uganda

Primer	Function	Sequence
EA 1.1	EcoRI-adapter	5' CTCGTAGACTGCGTACC 3'
EA 1.2	EcoRI-adapter	5' AATTGGTACGCAGTC 3'
MA 1.1	MseI-adapter	5' GACGATGAGTCCTGAG 3'
MA1.2	MseI-adapter	5' TACTCAGGACTCAT 3'
pre-EcoRI	Pre-amplification	5' GACTGCGTACCAATTC 3'
pre-MseI C	pre- & selective amplification	5' GATGAGTCCTGAGTAAC 3'
EcoRI AT	selective amplification	5' AGACTGCGTACCAATTCAT 3'
EcoRI GC	"	5' AGACTGCGTACCAATTCGC 3'
EcoRI CC	"	5' AGACTGCGTACCAATTCCC 3'

primers were used due to high polymorphism detected in previous studies (Okori *et al.,* 2003). The EcoRl selective primers were labelled with fluorescent dyes HEX or NED at the *5'* end of the primers. Each primer combination generated about 100-180 fragments, visualised as peaks. Two software, GeneScan AnalysisTM, 2.1 and Genotyper TM, version 2.0 (Perkin Elmer/Applied Biosystems, Foster City, CA. USA) were used to analyse the peaks. GeneScan was used to generate a data matrix in form of electropherograms from each gel run; while Genotyper generated a binary data matrix from GeneScan files as previously described (Okori *et al*., 2003; 2004b). In Genotyper, a manual and an automated procedure (using the skip overlap function), were used to define and score fragments. This process reduces the number of peaks generated by each primer combination leaving, only non-ambiguous fragments for data analysis.

Data analysis. Each AFLP fragment was considered as a single locus with two alleles, present or absent, and selectively neutral. In order to comparison changes in allele frequencies of the same DNA fragments in all populations, fragments were selected using the same categories. In Genotyper, the term category is equivalent to DNA fragment, and describes fragments visualised as peaks of a specific size (Perkin Elmer/Applied Biosystems, Foster City, CA. USA).

Data from primer combinations were analysed individually, or pooled. For the highland agroecological populations, totals of 75, 76 and 82 fragments were generated for primers EcoRI-AT, EcoRI-GC and EcoRl-CC, respectively. In the Lake Victoria Crescent, Masaka populations generated 56, 46 and 72 fragments and Wakiso, 65, 80 and 96 fragments for EcoRI-AT, EcoRl-GC and EcoRI-CC, respectively, at each location. For population genetic analyses, isolates from each year and location were treated as a population. Genetic analyses were performed at two levels, that is, at the same location, with a purpose of investigating temporal variations in genetic variability of populations over time. At the second level, analyses were performed to investigate temporal dynamics between populations from the two agroecologies. For the second level analysis, changes in allele frequencies of the same DNA fragments in all populations, was performed using one set of defined categories for each primer combination, for all populations. Thus, three category sets, one each for EcoRI-AT, EcoRl-GC and EcoRI-CC, were used and generated 75, 82 and 96 fragments, respectively.

Genetic variation in fungal collections was also measured by Nei's gene diversity, which measures the probability of obtaining two different alleles at a locus when two haplotypes are sampled from a population (Nei, 1973), and Nei's measure of genetic distance (Nei, 1987). Gene diversity is particularly suitable for asexually reproducing organisms or inbred populations such as *C. zeae-maydis* (Weir, 1996).

Statistical comparisons of gene diversity estimates were performed using t test (Nei, 1987). Genetic distances were computed using Popgene (Yeh and Boyle, 1997). A null hypothesis indicating presence of population structure between populations was analysed using Wright's population differentiation index F_{ST}. The population differentiation or fixation index is the reduction in heterozygosity expected with random mating at any one level of population hierarchy relative to another more inclusive level of hierarchy (Wright, 1978).

In this study, F_{ST} was estimated using the Analysis of Molecular Variance (AMOVA) framework (Weir and Cockerham, 1984; Excoffier *et al*., 1992), and implemented in Arlequin (Schneider *et al*., 2000). AMOVA estimates fixation indices in form phi (F) statistics that attempts to correct for the effects of sampling a limited number of organisms from a limited number of populations. Theoretically, the fixation index has a maximum of 1 and a minimum of 0, but as discussed by Hartl and Clark (1997), the observed maximum is usually less than 1 even in populations that are highly differentiated. Further tests for population differentiation were performed using exact tests at the 5% significance level (Raymond and Rousset, 1995), as implemented in Arlequin. Significance of ϕF_{ST} as estimated in AMOVA, was tested using non-parametric permutation procedures in Arlequin (Schneider *et al*., 2000).

RESULTS

Temporal dynamics in genetic variability within the same agroecology. Temporal analysis for population genetic differentiation revealed that *C. zeana* had no population structures between populations, within the same location (ϕF_{ST} = 0.05) (Table 2). This is indicative of lack of genetic structures in each agroecology. In all the annual populations, over 90% of variability was attributed to within population differences, rather than between populations. Populations from the two sites in the Lake Victoria Crescent and Kapchorwa highlands revealed a weak population structure (F_{ST} = 0.08, P = 0.01) (Table 2). In some cases, however, there were weak population structures between samples from different years (Table 3). Samples from the two districts in Lake Victoria Crescent, collected within the same year, were not genetically differentiated, indicating limited divergence due to microevolution induced processes. Exact tests did not provide support for population differentiation for populations (P> 0.05) from the same or different districts within either of the two agroecologies studied, i.e., Lake Victoria Crescent and Kapchorwa highlands. A further test for changes in inferred through analysis of polymorphism revealed a slight reduction in number of polymorphic fragments in subsequent populations in both agroecologies form the base year of the study (Table 4).

Estimates of gene diversity for populations from the same districts or districts within the same agroecologies were in the range of 0.1 to 0.22,

TABLE 2. Population differentiation of *Cercospora zeina* in three districts of Uganda based on analysis of molecular variance computed from multi-locus AFLP data sets

Source of variation	df	Variance components	Percentage of variation	ϕF_{ST} [a]
Individual districts				
[b]Masaka				
Among populations	3	1.03	5.93	
Within populations	266	16.37	94.07	
Total	269	17.40		0.05**
[b]Wakiso				
Among populations	2	0.92	*5.94*	
Within populations	197	14.61	94.06	
Total	199	15.53		*0.05***
[c]Kapchorwa				
Among populations	1	1.54	5.94	
Within populations	168	25.83	94.06	
Total	169	27.37		0.05**
[d]Districts within agroecology				
Among groups	1	0.67	3.57	0.08**
Populations within groups	5	1.02	5.43	
Within populations	455	17.13	91.01	
Total	461	18.82		

[a] = Significance tests based on 1000 permutations using Arlequin (Schneider *et al*., 2000). All analyses were performed according to Excoffier *et al*. (1992). Population refers to *C. zeina* isolates sampled during the same year and location

[b] = Samples collected from the Lake Victoria Crescent agroecological zone

[c] = Samples collected from the highland agroecological zone

[d] = Analyses were performed for populations from Wakiso and Masaka which belong to Lake Victoria Crescent agroecological zone

** Significant at P< 0.01

TABLE 3. Pair wise comparisons of ϕF_{ST} between populations of *Cercospora zeina* sampled at the same study sites from 2001 to 2003 in two agro-ecologies of Uganda

Individual districts	2001[a]	2001[b]	2002	2003			
[c]**Masaka**							
200la	0.00						
2001b	0.05*	0.00					
2002	0.04*	0.11*	0.00				
2003	0.05*	0.07*	0.04*	0.00			
[c]**Wakiso**							
2001	0.00						
2002	0.02*	0.00					
2003	0.07*	0.06*	0.00				
[d]**Kapchorwa**							
2002	-	-	0.00				
2003	-	-	0.05*	0.00			
[d]**Districts within agroecology**							
	Wak	Wak	Wak	Msk	Msk	Msk	Msk
	2001	2002	2003	2001[a]	2001[b]	2002	2003
Wak 2001	0.00						
Wak 2002	0.03*	0.00					
Wak 2003	0.06*	0.05*	0.00				
Msk 200la	0.08*	0.14*	0.11*	0.00			
Msk 2001'	0.09*	0.16*	0.12*	0.05*	0.00		
Msk 2002	0.06*	0.10*	0.10*	0.05*	006*	0.00	
Msk 2003	0.05*	0.07*	0.06*	0.07*	0.06*	0.05*	-

2001a and 2001b = long and short cropping seasons during which samples were collected; Wak = Samples from Wakiso, Msk = samples from Masaka; [c] = Lake Victoria Crescent agro-ecological zone. At Masaka samples were collected during the long and short cropping seasons of 2001. Subsequent sampling was only done during the long cropping season of each year during which the main maize crop is cultivated; [d] = Situated in the highland agro-ecological zone. Samples were taken at this sight, starting in 2002; ϕF_{ST} are analogues of Wrights F_{ST} computed according to Excoffier *et al.* (1992). Analysis is based on multi-locus data from all primer combinations used; and * = Significant at $P < 0.05$

and were not significantly different ($P \geq 0.05$); and in some cases decreased in succeeding populations from the base year of the study (Table 4). Pair-wise comparisons of Nei's genetic distances between populations from the same districts or districts within the same agroecologies confirmed only minor differences between populations (Table 5). However, in general, like gene diversity, the genetic distance between populations, from previous and subsequent years, were smaller than between populations separated by a year (Table 5).

Analysis of population genetic variability based on individual primer datasets generated similar parameters to the combined primer data set (data not shown). It revealed that most of the variability was generated by the selective primer combination, involving the primers *EcoRI* CC and *EcoRI*-GC. ϕF_{ST} ranged from 0.02 to 0.07, the highest being associated with *EcoRI*-CC. Gene diversity followed a similar trend, and was highest in the *EcoRI -CC/pre-MseI* C primer combination (0.25±0.12); and lowest with *EcoRI*-AT/pre-MseI C primer combination (0.11).

TABLE 4. Diversity indices generated from multi-locus AFLP data of *Cercospora zeina* populations sampled at the same study sites from 2001 to 2003 in two agro-ecologies of Uganda

	Number of fragments	Polymorphic fragments	[a]Average gene diversity[b]
[c]**Masaka**			
2001a	174	173	0.20 + 0.10
2001b	174	144	0.20 + 0.10
2002	174	133	0.16 + 0.08
2003	174	164	0.18 + 0.09
[c]**Wakiso**			
2001	241	223	0.12 + 0.06
2002	241	192	0.10 + 0.05
2003	241	192	0.14 + 0.07
[d]**Kapchorwa**			
2002	241	233	0.22 + 0.11
2003	241	227	0.22 + 0.11

2001a and 2000b refer to long and short cropping seasons during which samples were collected;
[a] = Average gene diversity computed according to Nei (1987) and Tajima (1983);
b = Gene diversity values are not significantly different at ($P < 0.05$). Tests were performed according Nei (1987);
[c] = Samples collected from the Lake Victoria Crescent agro-ecological zone; and
[d] = Samples collected from the highland agro-ecological zone

TABLE 5. Pair-wise comparisons of Nei's genetic distances between populations *of Cercospora zeina* sampled at the same study sites from 2001 to 2003 in two agro-ecologies of Uganda. Data were computed from a multi-locus data of all primer combinations used

	2001a	2001b	2002	2003
[c]**Masaka**				
200la 2001b	- 0.018	-		
2002	0.014	0.03		
2003	0.015	0.02	0.01	-
[c]**Wakiso**				
2001	-			
2002	-	0.05		
2003	-	0.02	0.011	-
[d]**Kapchorwa**				
2002	-	-	-	
2003	-	-	0.02	-

2001a and 2001b refer to long and short cropping seasons during which samples were collected;
[c] = Samples collected from the Lake Victoria Crescent agro-ecological zone; and
[d] = Samples collected from the highland agro-ecological zone staring in 2002

TABLE 6. Population differentiation of *Cercospora zeina* across three districts of Uganda based on analysis of molecular variance computed from multi-locus AFLP data sets

Source of variation	df	Variance components	Percentage of variation	ϕF_{ST} [a]
[b]Among groups	2	0.72	*3.56*	0.09**
[c]Among populations within groups	6	1.11	*5.50*	
Within populations	604	18.41	90.96	
Total	612	20.24		

[a] = Significance tests based on 1000 permutations using Arlequin (Schneider *et al.* (2000). All analyses were performed according to Excoffier *et al.* (1992). Population refers to *Cercospora zeina* isolates sampled during the same year and location;
[b] = Among groups = samples from the same district;
[c] = Populations within groups annual *C. zeina* samples taken at different locations of the same district; and
** Significant at P<0.01

TABLE 7. Pair wise comparisons of ΦF_{ST} between populations of *Cercospora zeina* sampled from different agroecologies of Uganda from 2001 to 2003

	Wak 2001	Wak 2002	Wak 2003	Msk 2001a	Msk 2001b	Msk 2003	Msk 2003	Kap 2002	Kap 2003
Wak 200l	0.00								
Wak 2002	0.03	0.00							
Wak 2003	0.06	0.05	0.00						
Msk 200l	0.08	0.13	0.13	0.00					
Msk 200la	0.09	0.16	0.12	0.04	0.00				
Msk 2002b	0.06	0.10	0.11	0.04	0.06	0.00			
Msk 2003	0.05	0.06	0.06	0.07	0.06	0.04	0.00		
Kap 2002	0.13	0.16	0.12	0.06	0.11	0.10	0.11	0.00	
Kap 2003	0.09	0.13	0.09	0.03	0.05	0.06	0.06	0.06	0.00

Wak = Samples from Wakiso, Msk = samples from Masaka, Kap = samples from Kapchorwa
200l and 2001[b] refer to long and short cropping seasons during which samples were collected
Analysis is based on multi-locus data from all primer combinations used. All ΦF_{ST} values were significantly different at P< 0.05

Temporal dynamics in genetic variability between agroecologies. Analysis of molecular variance revealed presence of a weak population structure over the study period for both ageoecologies (ϕF_{ST} = 0.09, P 0.01) (Table 6). Genetic differences between populations in the two study agroecologies, accounted for just over 3% of genetic variability found with the largest source of variability being due to populations from different districts. Exact tests were not significant (P> 0.05), indicating no population differentiation. Thus, when treated as an epidemiological unit, pairwise comparison of ϕF_{ST} showed weak to moderate genetic differentiation of populations from different districts that the two agroecologies (Table 7). We found that whereas districts are political management structures, effectiveness of agricultural extension systems and access to input and output markets vary. This variation, influences the types of maize varieties deployed and crop management processes used by farmers. This invariably moderates microevolution processes.

Populations from the same district with each agroecology had a weak or no genetic structure. Districts in general, will have similar cropping systems as suited for the agroecology and the absence of a population structure indicates. For

example, pair-wise ϕF_{ST} comparisons between Wakiso 2001 populations and Masaka and Kapchorwa 2003 populations had values of 0.05, and 0.09, respectively; compared with 0.06 and 0.13 in 2001 (Table 7). Pair-wise ϕF_{ST} comparisons also revealed that the Kapchorwa populations were more differentiated from the Wakiso or Masaka *C. zeana* (Table 7).

DISCUSSION

Temporal dynamics in genetic variability within the same agroecology

Variation within districts in the same agroecology. The objective of this study was to investigate temporal variation or dynamics in genetic variability of Ugandan *C. zeana* populations over a three-year period, starting from 2001 to 2003. The data show that within districts, in both agroecologies, no major difference in population structure within the same year or subsequent years was present ($\phi F_{ST} < 0.05$). The absence of a population structure districts is indicative of limited genetic divergence between populations. Within the same district, farmers generally deploy varieties that meet specific output markets demand as influenced by input market supply. Management practices will also be similar as influenced by extension systems and input markets. The net impact is that micro-evolutionary processes in the pathosystem will be attenuated similar factors. And, given the fact that epidemiological populations of *C. zeana* are clonal, but may be subject to microcycle microconidiation especially under stress (Lapaire and Dunkle, 2003), limited divergence is may occur. Between locations, however, there was evidence of a weak to moderate population differentiation, which was consistent throughout the study. Thus, the farming and or cropping systems may be driving the emergence of new pathotypes as is common in most agro-ecologies worldwide.

Variation between districts in the same agroecology. This study found evidence of a weak genetic structure between districts in the same agroecology over years suggesting a an effect of local selection. Maize production in the low land agroecology is based on open pollinated varieties, while in the highlands, the use of hybrids is most common. In these new materials, resistance to grey leaf spot is quantitative in nature (Coates and White, 1998; Sibiya *et al*., 2013; Nzuve *et al*., 2013; Berger *et al*., 2014). Quantitative resistance in general, does not create high selection pressure and ameliorates rates of microevolution of new pathotypes (McDonald and Linde, 2002). For a relatively new disease incited by a deuteromycete, the presence of a weak population structure, suggests that indeed there is a slow process of microevolution going on, hence the genetic weak genetic structure of the populations.

From a micro-evolutionary point of view, there are two possible explanations for these observations. First, evolutionary responsiveness of a pathogen, may be influenced by the numeric size of populations, as induced by gene flow and the mating system. Most pathogen populations during a cropping season follow a demographic cycle of rising and falling numbers (Burdon, 1993). This reduction in numeric size may attenuate random genetic drift, leading to population differentiation in the absence of geneflow (Lande, 1988; Whitlock, 1992; Whitlock and Burton, 1997; Futuyama, 1998). In Kapchorwa, maize production is under hybrids that are susceptible or moderately resistant to the disease. While in the Lake Victoria Crescent, production is generally under open pollinated varieties. In both agroecologies, conventional tillage is the practice of choice.

These differences in farming and cropping systems, affect pathogen demography and microevolutionary processes, accounting for differences between populations within each agroecology (Okori *et al.,* 2004a). These results illustrate the positive impacts of quantitative resistance deployment and the non-zero tillage based cropping systems used in the area. They corroborate with earlier findings that *Cercospora zeae-maydis* populations frequently undergo population numeric decrease that may influence not only epidemics (de Nazareno *et al*., 1993; Asea *et al.,* 2002), but also macroevolution as indicated in this study.

Temporal dynamics in genetic variability between agroecologies. Analysis of molecular variance revealed presence of a weak population structure over the study period, for both agroecologies ($\phi F_{ST} = 0.09, P<0.01$). However, genetic differences between populations in the two study agroecologies accounted for just over 3%. Nei's gene diversity, another measure of genetic differentiation, was small as is expected for asexually reproducing fungi but not significant. Gene diversity however, reduced in some younger populations, indicative of the effects of local selection pressure. Reduction in the number of polymorphic fragments in older populations, another measure of dynamics in these clonal populations, similarly, points to a possible role of local selection. The weak population structure over time between populations from the two agroecologies suggest that the two agro-ecologies may have pathosystems with very similar founder effects. Alternatively, there exists gene flow in form of movement of pathogen clones over long distances (Ward *et al.,* 1999).

Indirect estimates of gene flow (Nm) values were in the order of 2-10 in this study, providing strong support for the process. The interpretation of non-zero Nm values, particularly from old populations must, however, be treated with caution since the estimation procedure can generate Nm> 0 even when no individuals are exchanged between populations (Rousset, 2004).

Given that grey leaf spot of maize is a relatively new disease in sub-Saharan Africa (Ward *et al*., 1999), the non-zero Nm, show that pathogen movement between populations is frequent. In other pathosystems, such as the *Scierotinia sclerotiorum-canola* pathosystem and the barley-*Erysiphe graminis* pathosystem, geneflow flow promotes spatial mixing of clones, reducing the impact of selection (Kohn, 1994; Wolfe and McDermont, 1994). The net impact of gene flow is the cohesive evolution of species due to homogenisation of selectively adapted alleles (Morjan and Rieseberg, 2004). It is, therefore, logical, to presume that in spite of the existence of local selection and genetic drift in Uganda's *C. zeana* populations, the overriding effect of gene flow minimises the impact of these evolutionary forces resulting in slow population differentiation. Thus, from a management point of view, the source of new pathotypes is likely going to be due to local populations in unique agro-ecologies that may be spread easily by long distance transport.

The second interpretation of the data is related to carry-over effects of founder populations. In a similar study of the *wheat-Mycosphaerella graminicola* pathosystem in the USA, stability of field populations was attributed to large effective population size (Ne) and high genetic diversity of founder populations (Zhan *et al.*, 2001). In canola, founder effects of emigrant *Scierotinia scierotiorum* clones, from a recombining population, were detectable (Kohn, 1994). In previous studies, we (Okori *et al*., 2001; 2003) and other researchers (Dunkle and Levy, 2000 and Meisel *et al*., 2009), have shown that African *C. zeae-maydis* populations are predominantly constituted of *C. zeana* or Type II or sibling species, that is more genetically diverse than Type I common in the US and elsewhere. The primary asexual nature of *C. zeana,* and the fact that inoculum reduction is common (Ward *et al.,* 1999), suggests that the detected variability is conditioned by a genetically diverse founder population, and may partially account for the observations. Indeed, the experimental procedure used in this study, in principle, investigated changes in allele frequency of the same DNA fragments over time and, therefore, had capacity to capture any changes in genetic variability from founder populations. Data generated through this procedure suggest that microevolution may be promoting genetic homogeneity of a genetically diverse founder population, as indicated by small changes in genetic attributes. The continual role of founder populations, even after resistance, deployment may stem from the fact that in these agroecologies, some farmers still plant susceptible cultivars for various reasons, thus maintaining this pathogen diversity in such fields. Possible carry-over of the pathogen, especially by wind could contribute to population heterogeneity and override effects of local selection.

CONCLUSION

This study for the first time presents genetic studies on population dynamics of an important

pathogen of a major cereal in the tropics. The pathogen, *C. zeina,* like other necrotrophic fungi, undergoes cyclic annual demographic changes that impact on microevolution of the pathogen. Our data show that the net result of these changes, is a slow change in the population genetic structure that is influenced by agroecology farming and cropping systems, These findings imply that for grey leaf spot management in East Africa and many tropical areas where the disease is endemic, emergence of epidemic populations of *C. zeae maydis* can be minimised by regulated deployment of resistant genotypes, in combination with crop phytosanitation. Most of the material being deployed for the management of grey leaf spot today have quantitative resistance to the pathogen. This aspect will minimise the emergence of new pathotypes because of the slow selection pressure rendering greater protection and sustained maize productivity for the long term.

ACKNOWLEDGEMENT

This study was funded by the SIDA-SAREC East African Biotechnology Network (BlO-EARN) Project number 771799-771702. Additional funding was obtained from Nilsson-Ehle Foundation, Sweden.

REFERENCES

Agrios, G.N. 2005. Plant Pathology. 5th Edition. Academic Press.

Asea, G., Bigirwa, G., Adipala, E., Owera, S.A.P., Pratt, R.C. and Lipps, P.E. 2002. Effect of *Cercospora zeae-maydis* infested maize residue on progress and spread of grey leaf spot of maize in central Uganda. *Annals of Applied Biology* 140: 177-185.

Berger, D.K., Carstens, M., Korsman, J.N., Middleton, F., Kloppers, F.J., Tongoona, P., Alexander, A. and Myburg, A.A. 2014. Mapping QTL conferring resistance in maize to gray leaf spot disease caused by *Cercospora zeina. BMC Genetics* 15:60.

Bigirwa, G., Pratt, R.C., Adipala, E. and Lipps, P.E. 1999. Assessment of grey leaf spot and stem borer incidence and severity on maize in Uganda. *African Crop Science Conference Proceedings* 4:1-10.

Burdon, J.J. 1993. The structure of pathogen populations in natural plant communities. *Annual Review of Phytopathology* 31:305-323.

Burdon, J.J., Thrall, P.H. and Brown, A.H.D. 1999. Resistance and virulence structure in two Linum marginale-Melampsora lini host-pathogen metapopulations with different mating systems. *Evolution* 53:704-716.

Coates, S.T. and White, D.G. 1998. Inheritance of resistance to gray leaf spot in crosses involving selected resistant inbred lines of corn. *Phytopathology* 88:972-982

Crous, P.W., Groenewald, J.Z., Groenewald, M., Caldwell, P., Braun, U. and Harrington, T.C. 2006. Species of *Cercospora* associated with grey leaf spot of maize. *Studies in Mycology* 55:189-197.

Chupp, C. 1955. A monograph of the fungus genus Cercospora Ronald Press Co, Ithaca, New York, USA.

de Nazareno, N.R.X., Lipps, P. and Madden, L.V. 1993. Effects of corn residues on the epidemiology of gray leaf spot of corn in Ohio. *Plant Disease* 77: 67-70.

Dunkle, L.D. and Levy, M. 2000. The genetic relatedness of African and United States populations of *Cercospora zeae-maydis. Phytopathology* 90: 486-490.

Excoffier, L., Smouse, P. and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial restriction data. *Genetics* 131:479-491.

Futuyama, D.G. 1998. Evolutionary Biology. 3rd Edition. Sinauer Assiciates Sunderland Massachusetts.

Gomes, N.C.M., Fagbola, O., Costa, R., Rumjanek, N.G., Buchner, A., Mendona-Hagler, L. and Smaller, K. 2003. Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. *Applied and Environmental Microbiology* 69:3758-3766.

Gliessman, S.R. 1995. Sustainable agriculture: An agroecological perspective. *Advances Plant Patholology* 11:45-57.

Hartl, D.L. and Clark, A.G. 1997. Principles of Population Genetics 3rd Edition Sinauer

Associates Inc Sunderland, Massachusetts, USA.

Kohn, L.M. 1994. The clonal dynamic in wild and agricultural plant-pathogen populations. *Canadian Journal of Botany* 73:S1231-S1240.

Lamour, K.H. and Hausbeck, M.K. 2001. Investigating the spatial-temporal genetic structure *of Phytophthora capsici* in Michigan *Phytopatholgy* 91: 973-980.

Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455-1460.

Lapaire, C.L. and Dunkle, L.D. 2003. Microcycle conidiation in *Cercospora zeae-maydis*. *Phytopathology* 93:193-199.

Lederberg, J. 2000. Infectious history. *Science* 288:287-293.

Meisel, B., Korsman, J., Kloppers, F., Berger, D.K. 2009. *Cercospora zeina* is the causal agent of grey leaf spot disease of maize in southern Africa. *European Journal of Plant Pathology* 124: 577-583.

Milgoom, M.G. and Peever, T.L. 2003. Population biology of plant pathogens: the synthesis of plant disease epidemiology and population genetics. *Plant Disease* 87:608-617.

McDonald, B.A. and Linde, C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology* 40: 349-379.

Morjan, C.L. and Rieseberg, L.H. 2004 How species evolve collectively: Implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology* 13: 1341-1356.

Nei, M. 1973. Analysis of gene diversity in sub-divided sub-populations. *PNAS* 70: 3321-3323

Nei, M. 1987. Molecular Evolutionary Genetics New York: Columbia University Press, NewYork, USA.

Nzuve, F., Githiri, S., Mukunya, D.M. and Gethi, J. 2013. Combining abilities of maize inbred lines for grey leafspot (GLS), grain yield and selected agronomic traits in Kenya. *Journal of Plant Breeding and Crop Science* 5:41-47.

Okori, P., Fahleson, J. and Dixelius, C. 2001. Occurrence of gray leaf spot disease of maize in East Africa. *Fungal Genetics Newsletter* 48 (Suppi) 62.

Okori, P., Fahieson, J., Rubaihayo, P.R., Adipala, E. and Dixelius, C. 2003. Assessment of genetic variation among East African *Cercospora zeae-maydis* populations using AFLP and RFLP. *African Crop Science Journal* 11:75-86.

Okori, P., Rubaihayo, P.R., Adipala, E. and Dixelius, C. 2004a. Interactive effects of host, pathogen and mineral nutrition on grey leaf spot in Uganda. *European Journal of Plant Pathology* 110:19-128.

Okori, P., Rubaihayo, P.R., Fahleson, J., Adipala, E. and Dixelius, C. 2004b. Genetic characterisation of *Cercospora sorghi* from cultivated and wild sorghum and its relationship to other *Cercospora* fungi. *Phytopathology* 94:743-750.

Pratt, R.C., Lipps, P.E. and Freppon, J.T. 1997. Multi-disciplinary research on host resistance of maize to grey leaf spot. *African Crop Science Conference Proceedings* 3: 903-911.

Pratt, R.C. and Gordon, S.G. 2006. Breeding for resistance to maize foliar pathogens. *Plant Breeding Reviews* 26:119-173.

Provine, W.1986. Sewall Wright and Evolutionary Biology. University of Chicago Press Chicago, USA.

Raymond, M. and Rousset, F. 1995. An exact test for population differentiation. *Evolution* 49: 80-1283.

Robinson, R.A. 1987. Host management in crop pathosystems. Macmillan Publishers, London, UK.

Rousset, F. 2004. Inferences from spatial population genetics. In: Balding, D.J., Bishop, M. and Cannings, C. (Eds.). Handbook of Statistical Genetics. John Wiley and Sons Ltd, London, UK. pp. 681-712.

Schneider, S., Roessli, D. and Excoffier, L. 2000. Arlequin ver 2000: A software for population genetics data analysis: Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sibiya, J., Tongoona, P., Derera, J. and Rij, N. 2012. Genetic analysis and genotype X environment (G X E) for grey leaf spot disease resistance in elite African maize (*Zea mays* L.) germplasm. *Euphytica* 185: 349-362.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105:435-460.

Wang, J., Levy, M. and Dunkle, L.D. 1998. Sibling species of *Cercospora* associated with grey leaf spot of maize. *Phytopathology* 88:1269-1275.

Ward, J.M.J., Stromberg, E.L., Nowell, D.C. and Nutter, F.W.J. 1999. Grey leaf spot: A disease of global importance in maize production. *Plant Disease* 83:884-895.

Weir, B.S. 1996. Genetic Data Analysis II Sunderland Massachusetts. Sinauer Associates Inc Sunderland, Massachusetts, USA.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.

Whitlock, M.C. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution* 46: 608-615.

Whitlock, M.C. and Barton, N.H. 1997. The effective size of a subdivided populations. *Genetics* 146: 427-441.

Wolfe, M.S. and McDermont, J.M. 1994. Population genetics of plant pathogen interactions: The example of the *Erysiphe graminis-Hordeum vulgare* pathosystem. *Annual Review of Phytopathology* 32: 89-113.

Wortmann, C.S. and Eledu, C.A. 1999. Uganda's Agroecological Zones: A Guide for Planners and Policy Makers. Kampala, Uganda: Centro Internacional de Agricultura Tropical.

Wright, S. 1978. Evolution and the Genetics of Populations, University of Chicago Press, Chicago, USA.

Yeh, F.C. and Boyle, T.J.B. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgium Journal of Botany* 129: 157.

Zhan, J., Mundt, C.C. and McDonald, B.A. 2001. Using restriction fragment length polymorphisms to assess temporal variation and estimate the number of ascospores that initiate epidemics in field populations of *Mycosphaerella graminicola*. *Phytopathology* 91: 1011-1017.